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POST-PARTUM REGENERATION OF THE HUMAN ENDOMETRIUM

By ALBERT SHARMAN

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Modern knowledge of the post-partum regeneration of the human endometrium is based on the fundamental investigations of Friedländer (1870). He gave the first accurate description of the decidua with its compact and spongy layers. He also demonstrated that the mucosa in the non-placental region is restored by the fourth week but that of the placental site not until some weeks later. Subsequent articles on post-partum regeneration of the endometrium were published by Leopold (1877), Ries (1892), Werth (1895), Kroenig (1901), Wormser (1903), Teacher (1927), Schroeder (1930) and Williams (1931).

Williams, summarizing the main conclusions established by previous workers, stated that the restoration of the non-placental endometrium is practically completed during the third week, that the placental site is no longer evident in the sixth or seventh week and that mitoses have not been observed in the uterine epithelium before the third week. His own investigations were mainly concerned with the placental site. More recent writers have been concerned with endometrial evidence of the resumption of ovulation, but not with the histology of the restoration of the endometrium. Rutherford & Mezer (1942) studied both aspects of the subject and confirmed the earlier findings of the regeneration of the non-placental endometrium.

Previous work was limited in respect of the days covered. Teacher's study, for example, was of seven uteri from the second to the sixth week. Williams's classical contribution was mainly based on eighteen uteri obtained from the seventh day to the fourth month; while Rutherford & Mezer examined biopsy specimens on twenty-five occasions from the day of delivery until the fourteenth week.

The present investigation deals with the non-placental endometrium; but when uteri were obtained or when the appearance in biopsy specimens suggested that the specimens were derived from the placental region, the findings in that region were also noted. The study is the first investigation of endometrial regeneration in which the Unna-Pappenheim stain for plasma cells has been used. Some account of the restoration of the pre-gravid cyclical pattern is also given.

MATERIAL AND METHODS

The material included ten post-mortem uteri obtained as follows: two on the first day (day of delivery), one on the second day, one on the third day, one on the fourth day, one on the sixth day, three on the seventh day and one on the twelfth day. Another uterus was obtained by hysterectomy on the fifty-fourth day. A total of 626 biopsy specimens was obtained, by means of an endometrial biopsy curette, from 285 women from the fifth day to the ninth month. The material covered every day to the eighty-first; thereafter there were several specimens for each week until the end of the thirty-ninth week. The individuals from whom the biopsies were obtained had been delivered in hospital or in a nursing home in Glasgow; and all had been clinically free from puerperal infection.

The specimens were routinely fixed in corrosive-formol solution and embedded in paraffin and the sections stained with haematoxylin and eosin. Duplicate sections were stained by the Unna-Pappenheim method (methyl-green-pyronin) in order to facilitate the recognition of plasma cells.

The age distribution among the 285 patients was:

Under 20 years	10 (3·5 %)
20–29 years	154 (54 %)
30–39 years	111 (39 %)
40 years and over	10 (3·5 %)

Of the 285 women, 153 (54 %) were primiparae. One hundred and twenty were lactating at the time of their first biopsy; the other 165 were not lactating. One hundred and sixty-five biopsies were done in the lactating group, and 461 in the non-lactating group.

Menstruation began in approximately one-fourth of the cases within 6 weeks of delivery; 12 weeks after delivery 60 % of the cases had begun to menstruate. The general histological character of the endometrium in the 626 biopsies was as follows:

Proliferative	473 (147 in lactating women)
Secretory	153 (18 in lactating women)

All the secretory specimens were found to have been taken within 10 days of a supervening menstruation. Of the proliferative specimens thirty-two were found to have been taken within 10 days of a supervening menstruation. The proliferative group also includes specimens from fifteen patients who showed cystic glandular hyperplasia; a few of the latter were individuals who had repeat biopsies. It was found that normal proliferative and secretory patterns were later established. The hyperplasia occurred as early as the twenty-third day and as late as the ninth month post-partum.

Biopsy was usually made from the anterior uterine wall. Endometrial specimens from both the anterior and posterior walls were taken from those uteri examined at post-mortem. Several biopsies were taken from both walls of a few of the uteri which were examined at later dates post-partum. It was found that multiple biopsies showed almost identical appearances at each of these later stages.

DESCRIPTION

First day (*day of delivery—two post-mortem uteri*)

The first patient died two hours after delivery. On a complete cross-section of the uterus, in the non-placental area there is a lining of well-defined decidua containing many sinusoids. At the myo-endometrial junction there are numerous distended glandular spaces, lined by epithelium. The thickness of the endometrial lining varies between 0·8 and 1·4 mm. The entire endometrium is infiltrated with polymorphonuclear leucocytes and lymphocytes. Granules of blood-pigment are abundant in the endothelial cells of the sinuses and capillaries, in the young connective-tissue cells and in many of the polymorphonuclear leucocytes (pigmented phagocytes).

The placental site is occupied by a large, superficial blood-clot. In the deeper layer there are numerous dilated, thrombosed blood-vessels. Here occasional giant cells of the multi-nuclear type are also present.

The second patient died eighteen hours after delivery. Several endometrial specimens were taken from the anterior and posterior walls. The non-placental endometrium shows small blood vessels containing thrombi and, in a deeper layer, irregularly shaped glands. Degenerating decidual cells are recognizable but their outlines are becoming blurred and the nuclear staining is less distinct. Blood-pigment is abundant in the stromal cells and in the numerous polymorphonuclear leucocytes. The placental site shows fragments of syncytium, some hyaline degeneration of chorionic connective tissue and dilated vessels with thrombi. Giant cells are more numerous in the stroma than in the first specimen. Numerous polymorphonuclear leucocytes, lymphocytes and disintegrating red blood corpuscles are present (Pl. 1, figs. 1–3).

Second day (*post-mortem uterus*)

The non-placental area is covered with tangled decidual tissue without any surface epithelium. There are a few irregular gland-like spaces with no epithelium, small haemorrhages, thrombi and scattered lymphocytes. The decidual cells are smaller than in the earlier specimens, with occasional vacuolation, suggesting fatty transformation of the cells. In the placental site there is degenerating decidua with extensive blood-clot and thrombi, but fewer giant cells than in the previous specimen. Syncytial cells are rare. Between the decidua and muscle coat there are a few scattered lymphocytes and polymorphonuclear leucocytes (Pl. 1, figs, 4–6).

Third day (*post-mortem uterus*)

The endometrium in the non-placental region is still not covered by epithelium and varies in thickness between 0·5 and 1·0 mm. In it there are irregularly shaped spaces, usually lined either by endothelial-like cells or less frequently by cuboidal cells. Some of these spaces, however, have no epithelial lining, and, since they do not contain red blood cells, it is possible that they represent the remains of former uterine glands. Decidual cells are present but they are further diminished in size. The placental site is greatly reduced both in size and in thickness as compared with the first day. It is essentially similar in its histological characteristics to those of the first and second days, but syncytial cells and giant cells are absent (Pl. 2, figs. 7–9).

Fourth day (*post-mortem uterus*)

The endometrium of the non-placental site is still devoid of epithelium but now contains small clusters of glands, lined by cubical epithelium. There are still a few normal decidual cells, but others show hyaline necrosis. Considerable infiltration by polymorphonuclear leucocytes is still a feature. The placental site is characterized by the presence of extensive blood clots, demarcated on their basal aspects by numerous polymorphonuclear leucocytes and, occasionally, young fibroblasts. Most of the polymorphonuclear leucocytes are free of blood pigment. In the deeper layers irregular spaces with no epithelial lining are frequent. Between these spaces there are dilated blood vessels with thrombi. The intervening stroma is essentially similar in character to that of the non-placental site (Pl. 2, figs. 10, 11).

Fifth day (biopsy) and sixth day (post-mortem uterus)

Owing to the presence of blood clots in the superficial layer of the endometrium and thrombi in the dilated vessels of the deeper layer, it is concluded that the biopsy specimen taken from the anterior wall of the uterus represents the placental site. Unfortunately, no biopsy specimen was available of the posterior wall. In many respects the appearances are similar to those of the fourth day, but the hyaline necrosis of the decidual remnants is more advanced. There are also some well-formed glands in the deeper layer; these, in view of their appearance and situation, are most probably not new formations. An occasional giant cell is seen (Pl. 2, fig. 12). In the post-mortem specimen (sixth day), at the non-placental site, the endometrium still has no epithelial covering. Newly formed glands, lined by cubical epithelium, are occasionally seen. At the placental site superficial blood clots and, in the deeper layers, dilated blood vessels, filled with thrombi, give a characteristic appearance. In the deeper layers there are, again, irregular spaces with no epithelial lining: there are no giant cells and the hyaline necrosis is extensive (Pl. 3, fig. 13).

Seventh day (three post-mortem uteri and a biopsy)

The three uteri show very similar features. At the non-placental site some areas are covered by a definite surface epithelium. The stroma, although there are still occasional decidual cells present, exhibits for the first time the accepted characteristics of a normal endometrium. It is as yet a relatively thin layer, varying in thickness between 0·25 and 0·56 mm., with numerous superficially situated small glands and occasional distended glands in the deeper layers. Some of the latter even penetrate into the myometrium and a few of these contain polymorphonuclear leucocytes. Blood clots still form the main feature of the placental site, but these now are not only separated from the deeper layers by polymorphonuclear leucocytes and young fibroblasts, but have acquired a superficial covering of a similar nature. In the deeper layers of the endometrium, dilated and thrombosed vessels are numerous. A few dilated glands with flattened epithelium can be seen. In the stroma there are occasional areas of necrotic decidua and some healthy decidual cells. In the biopsy specimen (placental site) there are dilated, thrombosed blood vessels, surrounded by stratified layers of decidual tissue, infiltrated by many polymorphonuclear leucocytes and lymphocytes. Most of the decidual cells are now almost completely hyalinized (Pl. 3, figs. 14–16).

Eighth day (biopsy)

The appearance of the specimen (absence of blood clots and thrombosed blood vessels) suggests that the biopsy represents the non-placental site. Numerous well-formed glands are present in a typical cellular stroma, which has a well-formed columnar surface epithelium. A few mitotic figures are seen, for the first time, in the glandular epithelium. There are still considerable numbers of normal decidual cells, some showing active cell division, suggesting that these survivors may become incorporated in the regenerated endometrium. Blood pigment or pigment containing polymorphonuclear leucocytes are absent in this and in all subsequent specimens. Lymphocytes are now replacing the polymorphonuclear leucocytes (Pl. 3, fig. 17).

Ninth and tenth days (two biopsies)

In the non-placental area, in both specimens, the surface epithelium is now well formed in places, and it appears to be derived from the dividing cells of the glandular epithelium. In the cellular stroma occasional clusters of hyalinized decidual cells are still present. Polymorphonuclear leucocytes are much fewer and chiefly confined to these areas with hyalinized cells. Lymphocytic infiltration appears to be increasing and for the first time an occasional plasma cell is seen. At the placental site, in both specimens, the organization of the superficial blood clot is more advanced as compared with earlier specimens. There is more marked invasion by young fibroblasts. The stroma of the deeper layers is becoming cellular and the gland spaces are lined by flattened or cubical epithelial cells. Occasional areas of hyalinized decidual tissue can be seen (Pl. 3, fig. 18; Pl. 4, figs. 19, 20).

Eleventh day (biopsy)

In the non-placental endometrium the surface epithelium is well formed. Some well-formed glands of the early proliferative phase are present, but others are less mature in appearance. Mitotic figures are seen both in the glandular and in the surface epithelium. A small area of decidual tissue is present. Polymorphonuclear leucocytes are further reduced in number, while plasma cells are becoming more numerous (Pl. 4, fig. 21).

Twelfth day (post-mortem uterus and a biopsy)

The biopsy specimen appears to be from the non-placental site. The surface epithelium continues to extend. The glands in many places are normal and numerous mitoses in the epithelium are present. Decidual tissue showing advanced hyalinization is conspicuous. Lymphocytic and plasma cell infiltration is present and there is a moderate number of polymorphonuclear leucocytes. The post-mortem uterus exhibits similar features in the non-placental site: unfortunately in this specimen post-mortem changes made detailed histological observations difficult. The thickness of the non-placental endometrium varies from 0·2 to 0·4 mm.

Thirteenth day (two biopsies)

In one of the patients biopsy was done of both the anterior and posterior uterine walls. The anterior, non-placental wall, shows very good surface epithelium of the low-cubical type. The glands, also, are well formed but still not as numerous as in the endometrium of a typical early proliferative phase. Hyalinization of decidual remains continues and there are very few polymorphonuclear leucocytes. The posterior, placental wall, contains degenerate decidua only, with very many lymphocytes and a few plasma cells. Biopsy of the second patient shows degenerate decidua only, the appearances suggesting that it represents the placental site.

Fourteenth and fifteenth days (two biopsies)

In the non-placental area, in both specimens, the epithelial covering is well formed. Some well-formed glands typical of the early proliferative phase are present, but others are still rudimentary. A little hyalinized tissue, obviously decidual but without recognizable cell outlines, contains a few polymorphonuclear leucocytes (Pl. 4, fig. 22). Lymphocytic infiltration is now a feature. Plasma cells are present in large numbers in the fourteenth-day specimen but are absent from the fifteenth-day specimen.

Sixteenth day (biopsy)

In the non-placental site proliferative endometrium devoid of decidual cells is present with complete surface epithelium. Restoration to normal proliferative endometrium is virtually complete at this stage.

Seventeenth day to twenty-second day (nine biopsies)

From the seventeenth to the twenty-second days the glandular pattern is of the normal proliferative type. Degenerating or metamorphosing decidual cells are seen in most of the biopsies. The stroma shows scattered plasma cells and areas of lymphocytic infiltration. Polymorphonuclear leucocytes are present in only three specimens.

Twenty-third day to twenty-eighth day (fourteen biopsies)

The glandular pattern is of the normal proliferative type. Areas of degenerating decidual cells are present in only three of the biopsies. Although cellular infiltration is appreciably reduced, nevertheless, polymorphonuclear leucocytes were observed in two, and lymphocytes and plasma cells in five specimens.

Twenty-ninth day to sixtieth day (191 *biopsies*)

From the twenty-ninth to the forty-ninth days the glandular pattern is of the typical proliferative type in all but eight specimens. A secretory pattern, with typical serrated glands, presumptive evidence of ovulation, was seen on the forty-fourth day; and specimens from seven other non-lactating women also gave secretory stages in the seventh week (Pl. 4, fig. 23). The stroma is infiltrated with plasma cells and lymphocytes in all but a few specimens. In six of the hundred biopsies degenerate decidual cells are still present. From the fiftieth to the sixtieth days a completely repaired endometrium is found, in some instances secretory and in others proliferative. In only three instances are decidual remnants, surrounded by concentrations of plasma cells and lymphocytes, present. In the remaining material, stromal infiltration is present in about one-half of the specimens. The uterus of the fifty-fourth day shows that the entire endometrium is completely restored (Pl. 4, fig. 24).

Sixty-first day to ninth month (400 *biopsies, ninety-two patients*)

The glandular and stromal pattern has completely returned to that of the typical non-gravid state, showing both proliferative and secretory stages, with the exception that stromal infiltration by appreciable numbers of lymphocytes and plasma cells is present in 120 of the 400 endometria examined (30 %). Fifty of the 135 (37 %) of the endometria during the third and fourth months showed this infiltration. However, after the fourth month, plasma cells and lymphocytes were seen only occasionally and in small numbers. Of the 400 endometria, 264 showed proliferative features and 136 secretory.

DISCUSSION

On a survey of all the findings a picture is obtained of two principal histological processes: (1) the regeneration of the endometrium, and (2) the 'inflammatory' reaction. These will be discussed separately; and attention will be drawn to new observations.

During the first week the main features are portions of degenerating decidua with blood clot in varying degrees of organization, thrombosed blood vessels and glands of varying size, some dilated and many showing proliferation of the epithelium, the cells of which are irregular in shape and size. The decidual tissues occupy a more superficial position and the glands a deeper one. Giant cells are not seen after the fifth day (with the exception of an occasional one seen in one of the specimens of the seventh day). Mossman (1937) states that these giant cells are probably derived from the mononucleate decidual cells and that they disappear immediately post-partum. My findings agree with those of Mossman. Blood pigment in the tissue spaces and in the phagocytes is not seen after the seventh day. In the second week restoration advances quickly and a proliferative mucosa is recognizable in the sections of the eighth and ninth days, although scattered elements of decidual remains are present.

Examination of this series has revealed the early appearance on the eighth and ninth days of mitotic figures in the glandular epithelium, with the resulting development of an almost complete surface epithelium by the end of the second week. These proliferative changes, showing active cell division, probably go on uninterrupted until the reparative processes are completed, and thereafter at a slower rate until follicular maturation commences. Fully restored endometrium is seen in all specimens examined from the sixteenth day onwards. The endometrium has now been repaired except in respect of (1) hyalinizing decidual remnants and (2) the cellular infiltration of the stroma. Attention should be drawn to the persistence of these decidual cells in the stroma, and moreover, to their occasional active cell division. It seems very probable that these surviving decidual remnants become incorporated in the future endometrium, after they have reverted to the connective-tissue type. It would appear, therefore, that the decidua is not completely shed with the placenta and membranes at parturition or in the lochia of the puerperium, as has been the accepted teaching, but that many of these cells are utilized in the reconstruction of the endometrium. This is not surprising if Mossman's (1937) definition of decidual cells is borne in mind: 'They are endometrial connective-tissue cells which have become rounded or polyhedral due to the storage of glycogen or lipoids in their cytoplasm.' It has already been shown that the presence of decidual cells after the sixth week is rare.

As recorded above, the earliest presumptive evidence of ovulation was the secretory endometrial pattern obtained on the forty-fourth day; and seven other non-lactating women also gave secretory endometrial specimens in the seventh week. There is considerable variation in the statements in the literature as to the duration of post-partum anovularity. The present observations indicate that the pre-gravid cyclical pattern is not restored before the end of the sixth week.

In discussing the significance of the frequent cellular infiltration of the stroma, it may be recalled that in the normal menstrual cycle there are almost always some lymphocytes but at no time are there plasma cells in the endometrium. A few polymorphonuclear leucocytes can be seen premenstrually from about 2 days before the onset of menstruation. It has been shown above that there is, immediately post-partum, a leucocytosis, manifesting itself in a very large number of polymorphonuclear leucocytes and lymphocytes, which persists for about 10 days. This would seem to be a defensive process, anti-bacterial and designed to allow the normal mechanism of repair to proceed. During the first 10 days the numbers of polymorphonuclear leucocytes appear to diminish progressively; after this date polymorphonuclear leucocytes are seen only occasionally and in small numbers. After the tenth day plasma cells enter the scene.

Thus the so-called 'endometritis' in the reparative days of the puerperium conforms to the normal process of tissue repair and is not pathological but physiological. It is more difficult to explain the prolonged persistence of plasma cell and lymphocytic cell infiltration, except of course when necrotic decidua is present. As late as the third and fourth months, as already stated, 37 % of the endometria examined showed infiltration. Examination of my specimens shows conclusively that lymphocytes and plasma cells normally persist for several months in the post-partum endometrium. Stromal infiltrations of plasma cells and lymphocytes, hitherto held to imply endometrial infection, need not carry that implication. They may instead indicate a recent pregnancy; after the sixth week post-partum they may, in fact, constitute the sole indication. These observations are, in part, supported by Dumoulin & Hughesdon (1951), who, in their study of seven normal post-partum cases, found plasma cells in the endometrium in one case on the sixteenth day and in five cases at the sixth week.

There can be little doubt that, in general, endometrial reparative processes are under the direct control of the ovaries. But opportunities (e.g. bilateral oophorectomy soon after parturition) rarely offer themselves for clinical observation on this point. In this connexion, one patient in the series under study is of interest. Three months after delivery she had a curettage of the uterus and bilateral oophorectomy. The endometrium was of the normal secretory pattern. Thereafter, ten endometrial biopsies were performed at weekly intervals. The first four showed scanty proliferative endometrium, the fifth showed only one small proliferating gland while the remaining five yielded no endometrium whatsoever. It was apparent that the removal of the ovaries had resulted in an almost complete cessation of endometrial repair.

SUMMARY

1. A study of the post-partum repair of the non-placental endometrium was based on eleven uteri and on 626 endometrial biopsies. The biopsy specimens were obtained from 285 women from the day of delivery (first day) to 9 months after parturition.

2. Mitotic figures in the gland-epithelium were first seen on the eighth day. The endometrium was structurally restored in all specimens from the sixteenth day onwards.

3. Decidual cells in the stroma were found to undergo active cell division from the eighth day and later to become incorporated in the future endometrium,

reverting ultimately to connective-tissue type; they were rarely present after 6 weeks.

4. Stromal infiltration, with plasma cells and lymphocytes in appreciable numbers, was found in about 50 % of biopsies at 6 weeks post-partum and in 37 % at 3–4 months; and was even found at later months.
5. The first secretory pattern was seen on the forty-fourth day.

This work was commenced at the suggestion of Prof. W. J. Hamilton. My thanks are due to Dr P. Bacsich for advice and assistance in histological interpretation and to Mr G. Marshall for the microphotographs.

REFERENCES

Dumoulin, J. G. & Hughesdon, P. E. (1951). Chronic endometritis. *J. Obstet. Gynaec.* 58, 222–235.

Friedländer, C. (1870). *Physiol.-anat. Untersuchungen über den Uterus.* Leipzig.

Kroenig, J. (1901). Beitrag zum anatomischen Verhalten der Schleimhaut der Cervix und des Uterus während der Schwangerschaft und im Früwochenbett. *Arch. Gynaek.* 63, 26–37.

Leopold, G. (1877). Studien über die Uterusschleimhaut während Menstruation, Schwangerschaft und Wochenbett. *Arch. Gynaek.* **11**, 443–500.

Mossman, H. W. (1937). Comparative morphogenesis of the fetal membranes and accessory uterine structures. *Contr. Embryol. Carneg. Instn*, **158**, 133–246.

Ries, E. (1892). Klinische und anatomische Studien über Zurückbildung des puerperalen Uterus. *Z. Geburtsh. Gynäk.* **24**, 33–89.

Rutherford, R. N. & Mezer, J. (1942). Regeneration of uterine mucosa after spontaneous abortion and normal delivery. *J. Amer. Med. Ass.* **119**, 124–128.

Schroeder, R. (1930). The uterus in the puerperium. In Moellendorff's *Handbuch der Mikroskopischen Anatomie des Menschen*, pp. 329–556. Berlin: J. Springer.

Teacher, J. H. (1927). On the involution of the uterus post-partum. *J. Obstet. Gynaec.* **34**, 1–27.

Werth, R. (1895). Untersuchungen über die Regeneration der Schleimhaut nach Ausschabung der Uteruskörperhöhle. *Arch. Gynaek.* **49**, 369–470.

Williams J. W. (1931). Regeneration of the uterine mucosa after delivery, with especial reference to the placental site. *Amer. J. Obstet. Gynaec.* **22**, 664–696.

Wormser, E. (1903). Die Regeneration der Uterusschleimhaut nach der Geburt. *Arch. Gynaek.* **69**, 449–579.

EXPLANATION OF PLATES

Microphotographs stained by haematoxylin and eosin

Plate 1

Fig. 1. Section through non-placental endometrium of uterus 2 hr. post-partum: distended glandular spaces (surviving glands) are present at the myo-endometrial junction. × 10.

Fig. 2. Section through non-placental endometrium of uterus 2 hr. post-partum: large areas of decidual cells are infiltrated by polymorphonuclear leucocytes: sinusoids and capillaries are filled with red blood-corpuscles and pigmented and non-pigmented polymorphonuclear leucocytes. × 50.

Fig. 3. Section through non-placental endometrium of uterus 2 hr. post-partum, showing the superficial lining of the uterine cavity; it contains well-defined decidual cells, and is loaded with polymorphonuclear leucocytes and lymphocytes. × 450.

Fig. 4. Non-placental endometrium 26 hr. post-partum: gland-like spaces, with no epithelium and irregular in size and shape, are seen superficially. × 50.

Fig. 5. Non-placental endometrium 26 hr. post-partum: typical regressing decidual cells are shown. × 450.

Fig. 6. Non-placental endometrium 26 hr. post-partum: gland-like spaces, irregular in size and shape and with no epithelium in many, are present. × 450.

PLATE 2

Fig. 7. Non-placental endometrium 67 hr. post-partum: the tissue-spaces, which are probably the remains of former glands, crushed and de-epithelialized during pregnancy, are conspicuous. × 50.

Fig. 8. Non-placental endometrium 67 hr. post-partum: decidual cells, faintly staining and losing their cell outlines; note infiltration by polymorphonuclear leucocytes and lymphocytes. × 200.

Fig. 9. Non-placental endometrium 67 hr. post-partum: some of the tissue spaces are lined with flattened cells almost endothelial in appearance; in some places these cells are almost low-cubical; some of the spaces have no epithelium. × 200.

Fig. 10. Non-placental endometrium 4 days post-partum: hyalinization of decidual remnants. × 50.

Fig. 11. Non-placental endometrium 4 days post-partum: the decidual cells are losing their characteristics and many are acquiring the appearance of young fibroblasts; polymorphonuclear leucocytes are numerous. × 450.

Fig. 12. Placental endometrium 5 days post-partum: these well-formed glands, seen in a deeper layer, are survivals and not new glands. × 450.

PLATE 3

Fig. 13. Non-placental endometrium 6 days post-partum: the fibroblastic conversion of decidual cells is apparent; a few polymorphonuclear leucocytes are seen in a new gland. × 450.

Fig. 14. Non-placental endometrium 7 days post-partum: glands and stroma are well-defined. × 30.

Fig. 15. Non-placental endometrium 7 days post-partum: the epithelium lining this gland is well-formed; most of the decidual cells are completely hyalinized; their fibroblastic conversion continues. × 350.

Fig. 16. Non-placental endometrium 7 days post-partum: glands are beginning to show an epithelial lining. × 350.

Fig. 17. Non-placental endometrium 8 days post-partum: mitosis (marked by arrow) in the glandular epithelium is seen for the first time post-partum. × 350.

Fig. 18. Non-placental endometrium 9 days post-partum: a well-formed surface epithelium, a normal gland and a large thrombosed vessel are to be noted; the area enclosed in square is shown at a higher magnification on Pl. 4, fig. 19. × 50.

PLATE 4

Fig. 19. Non-placental endometrium 9 days post-partum: mitoses (indicated by arrows) are present in the gland-epithelium on the left; the glandular epithelium on the right will become surface epithelium. × 450.

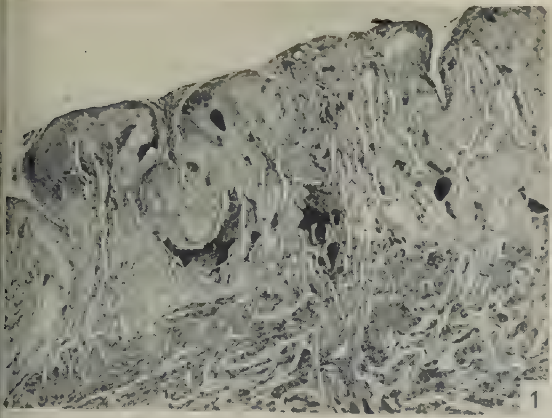
Fig. 20. Non-placental endometrium 10 days post-partum: note mitoses in gland epithelium. × 350.

Fig. 21. Non-placental endometrium 11 days post-partum: note well-formed glands of proliferative type. × 350.

Fig. 22. Non-placental endometrium 15 days post-partum: fibroblastic metamorphosis of decidual cells is conspicuous. × 350.

Fig. 23. Endometrium on 48th day. × 50. Note characteristic secretory (ovulatory) appearances: menstruation started later on the same day.

Fig. 24. Cross-section of a uterus on 54th day. The entire endometrium is restored to normal. × 20.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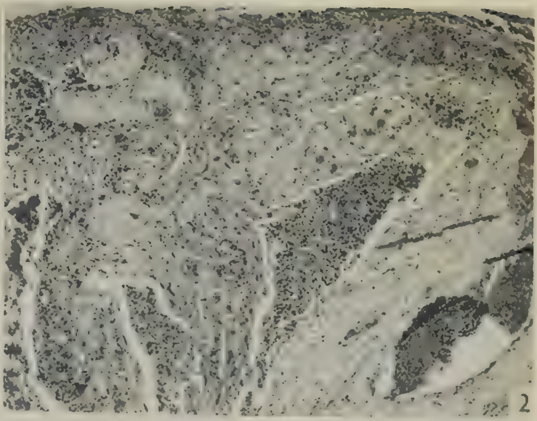
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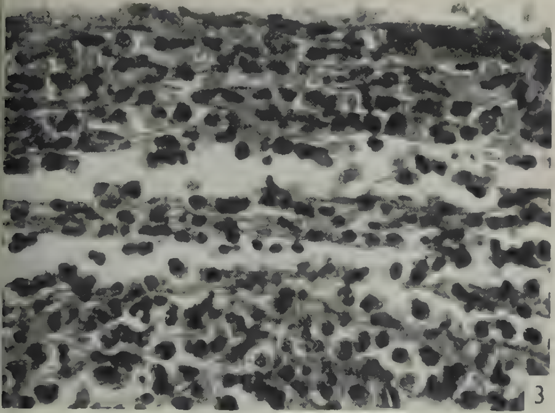
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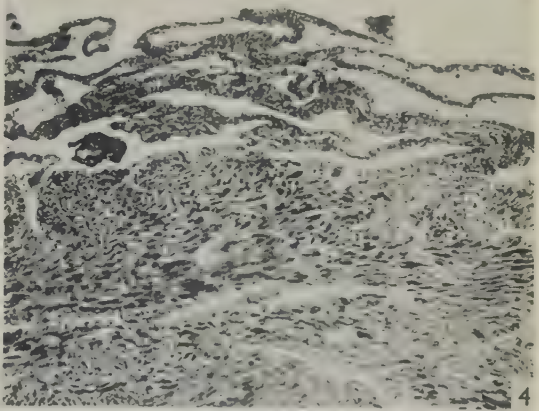
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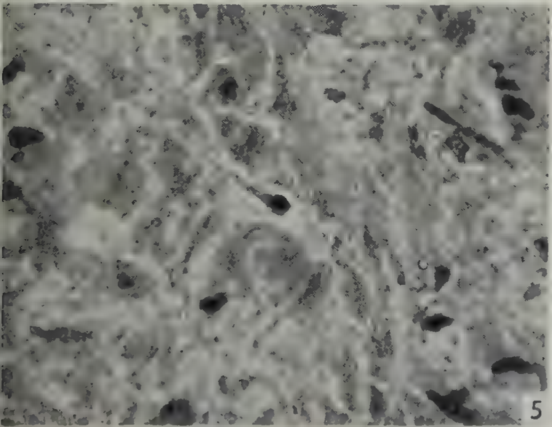
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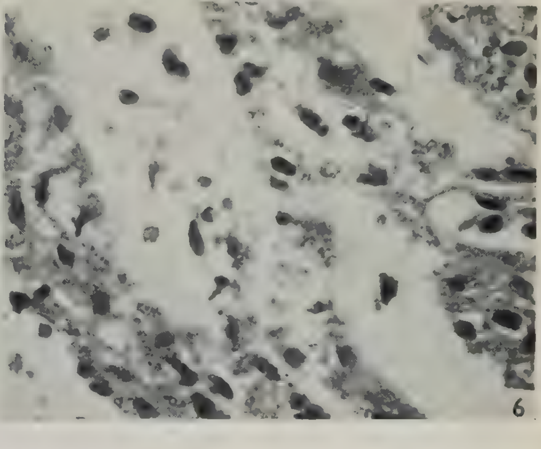
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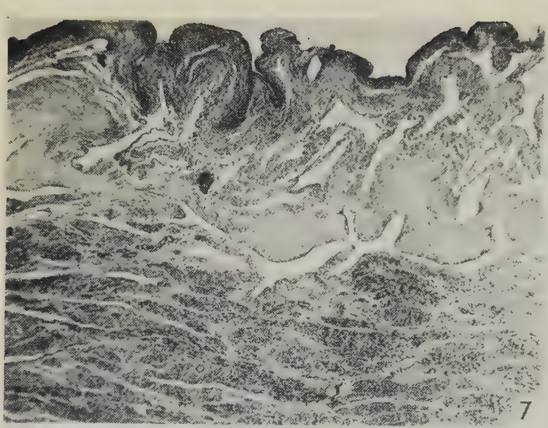
SHARMAN—POST-PARTUM REGENERATION OF THE HUMAN ENDOMETRIUM



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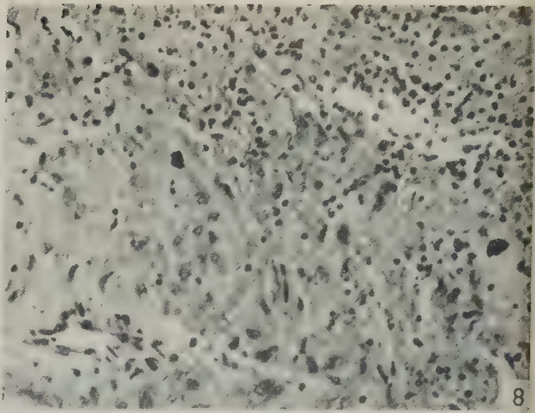

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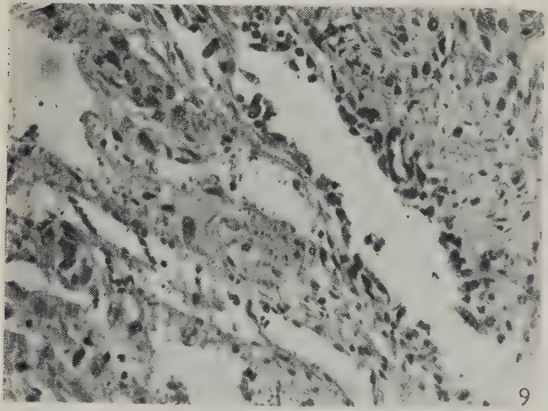

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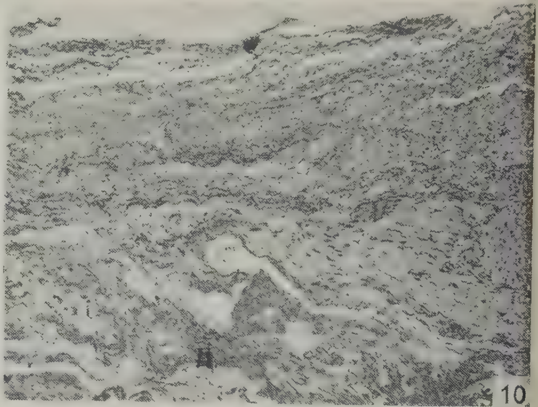

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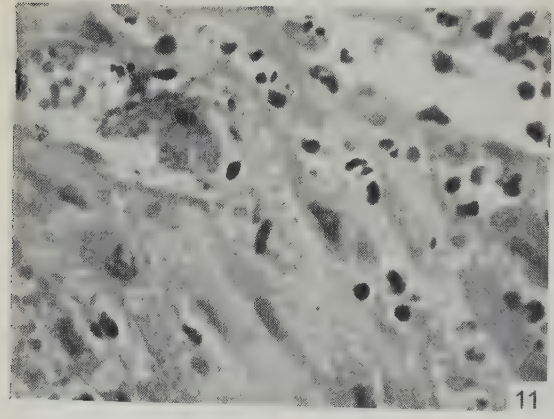

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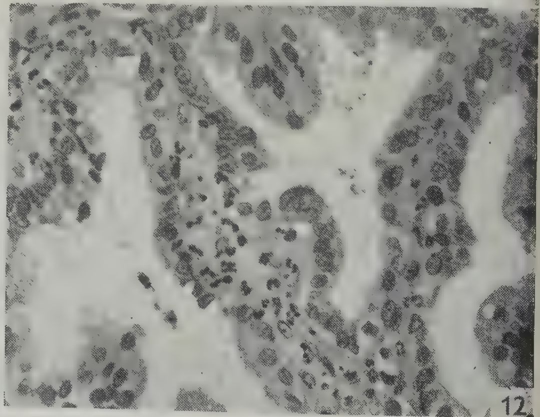

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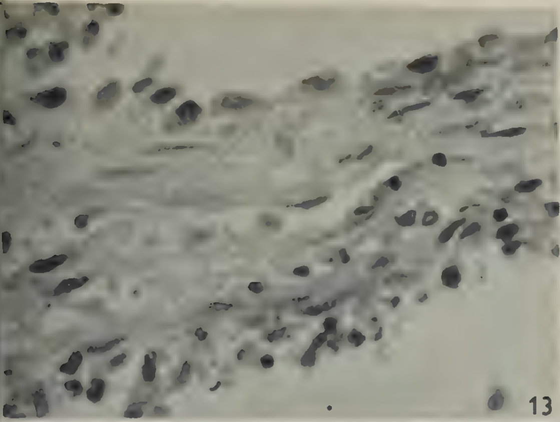
SHARMAN—Post-partum regeneration of the human endometrium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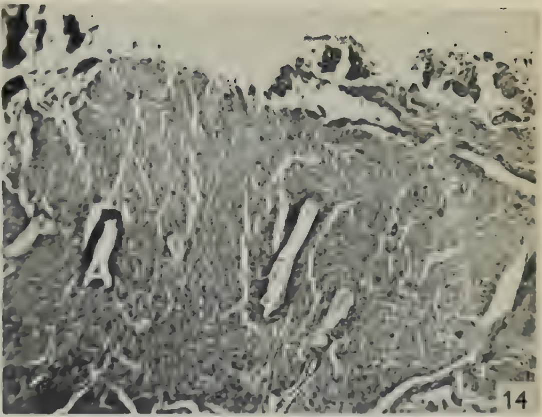
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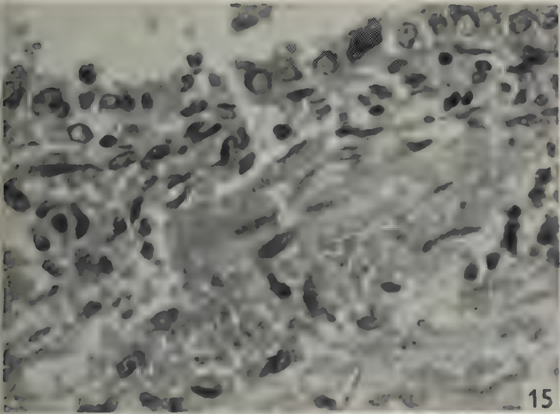
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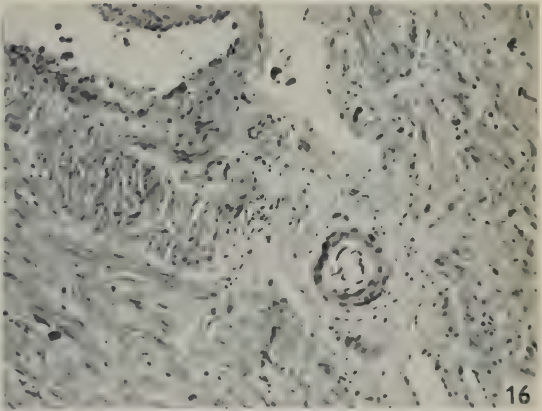
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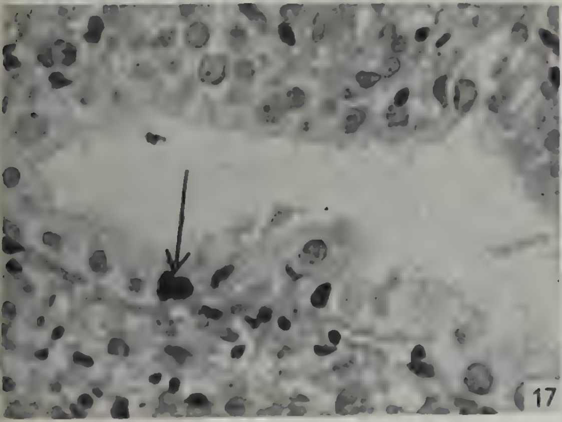
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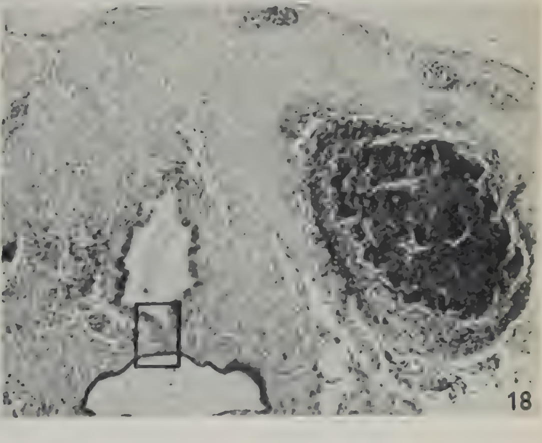
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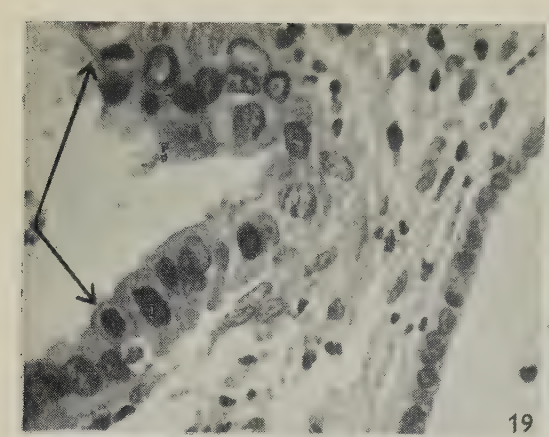
SHARMAN—POST-PARTUM REGENERATION OF THE HUMAN ENDOMETRIUM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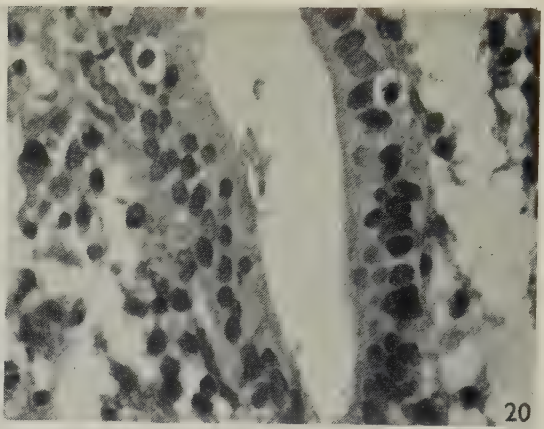
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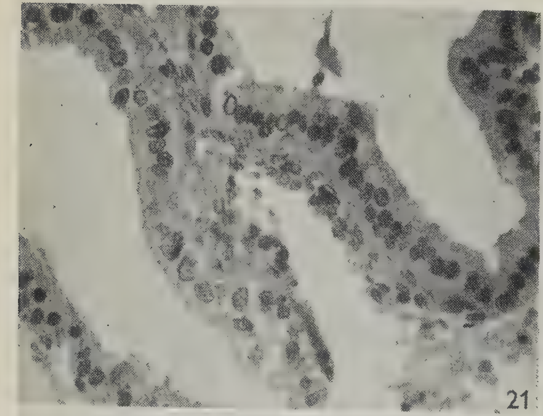
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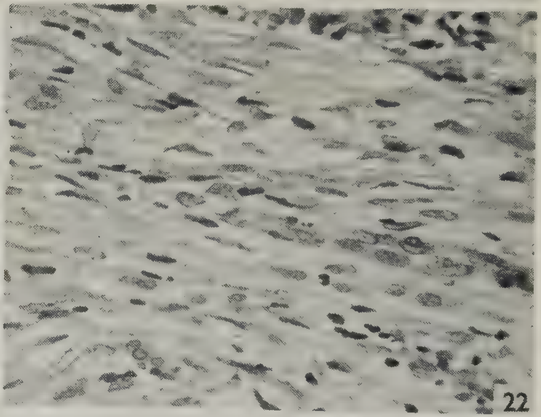
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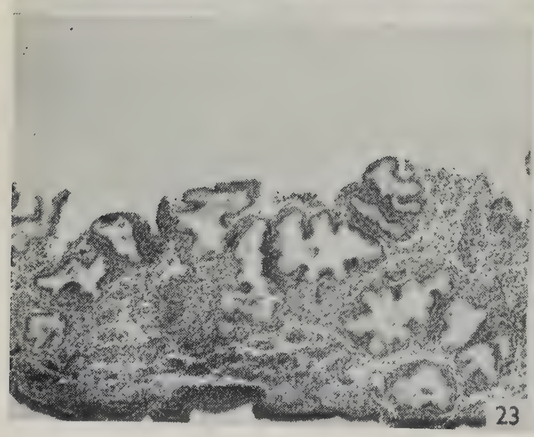
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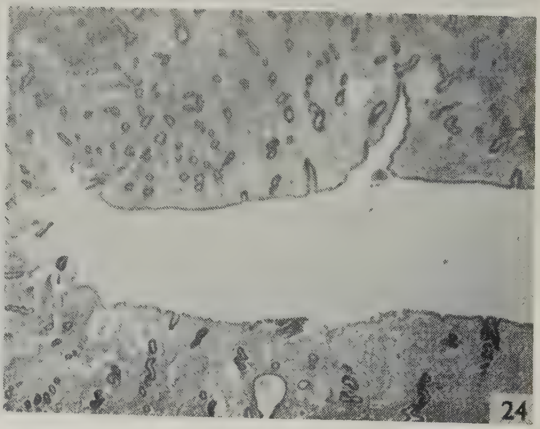
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SHARMAN—POST-PARTUM REGENERATION OF THE HUMAN ENDOMETRIUM

THE ROTATORY MOBILITY OF THE FIBULA IN EUTHERIAN MAMMALS

By C. H. BARNETT and J. R. NAPIER

Department of Anatomy, St Thomas's Hospital Medical School

It is generally recognized that the rotatory mobility of the fibula varies considerably throughout the eutherian mammals. Little functional significance has been assigned to this variation, however, apart from the observation of Carleton (1941) that immobility of the fibula is characteristic of jumping, burrowing and swimming mammals, while mobility is confined to certain members of the Carnivora and the Primates.

Walmsley (1918) came to the conclusion that the proximal end of the fibula existed mainly as a muscular process, while the distal end was the significant portion which owed its existence in a mobile form to the movements of the foot. Owen's view (1866) was similar, though more specific, when he stated 'it is to this prehensile power [of the foot] that the modifications of the fibula chiefly relate'. The authors (1952) have shown that, in man, the mobility of the fibula, as judged by the criteria of the shape, inclination and size of the superior tibio-fibular joint, is related to movement at the ankle and hence to the form of the talus. The fibula is mobile in those individuals in whom the axis of rotation at the ankle joint is steeply inclined, but is relatively immobile if this axis is horizontal.

This finding in man suggests a method of approach to the wider problem of the functional significance of fibular mobility in other mammals. In the present investigation an attempt has been made to link mobility of the mammalian fibula with movements at the ankle during locomotion.

MATERIALS AND METHODS

Ninety-four species, comprising representatives from all the known orders of eutherian mammals with the exception of the Cetacea, Sirenia and Dermoptera, and including most of the important families, were examined. The specimens were, for the most part, in the form of disarticulated limb bones, but at least one wet specimen from each of the more common orders and suborders was dissected; these amounted to twenty-four in all and are indicated in Tables 1–3. Where rotatory mobility was anticipated the bones were stripped of all their muscles, leaving a ligamentous preparation of the two limb bones with the attached foot. Metal indicator pins were driven into each long bone and the relative divergence of these two pointers during passive movement, indicating rotation of the fibula relative to the tibia, was photographed and measured. This rotation is usually extremely small, and it may easily be missed unless such a procedure is adopted.

Measurements of the tali were made in accordance with the method employed for the human talus. The axis of the ankle joint was ascertained by finding central points of the circles of which the curvatures of the medial and lateral profiles of the talus form a part.

OBSERVATIONS

The majority of fibulae could be classified according to the characteristics of the superior and inferior tibio-fibular joints into three distinct groups: the immobile, the mobile and the intermediate.

(1) *The immobile fibula*, partly or wholly fused to the tibia, is widely represented among the mammals, and examples were found in all the orders examined, although the tarsier, *Tarsius spectrum*, is unique among the Primates (Table 1). There are two main varieties within this group: first, those showing bony fusion of the tibia and fibula at the upper end associated with fusion or an extensive fibrous tissue union at the lower (Figs. 1 and 2); and, secondly, those showing fusion at the lower end with a synovial joint at the upper (Fig. 3). Both types are associated with a talus the appearance of which is generally rather square, there being no splaying of the medial and lateral sides (Fig. 4A). The two profiles of the trochlear surface of the bone often show symmetrical and equal curvatures, so that the axis of movement at the ankle tends to be parallel to the plane of the trochlear surface and a true ginglymoid movement results.

Table 1. *The immobile fibula*

(Where wet specimens have been examined they are indicated by an asterisk.)

Animal	Order	Superior tibio-fibular joint	Inferior tibio-fibular joint
**Erinaceus europaeus* (hedgehog)	Insectivora	Fused	Fused
Gymnura gymnura (gymnura)	Insectivora	Fused	Fused
**Sorex araneus* (common shrew)	Insectivora	Synovial	Fused
Desmana moschata (Russian desman)	Insectivora	Fused	Fused
Potamogale velox (otter shrew)	Insectivora	Synovial	Fused
Macroscelides proboscideus (common elephant shrew)	Insectivora	Synovial	Fused
Petrodromus tetradactylus (four-toed elephant shrew)	Insectivora	Fused	Fused
Rhynchocyon cirnei (long-nosed jumping shrew)	Insectivora	Fused	Fused
Centetes ecaudatus (tenrec)	Insectivora	Synovial	Fused
**Chrysochloris asiatica* (cape golden mole)	Insectivora	Fused	Fused
Halichoerus grypus (grey seal)	Carnivora	Fused	Syndesmosis
Otaria californiana (sea-lion)	Carnivora	Fused	Syndesmosis
Procavia capensis (Cape coney)	Hyracoidea	Fused	Fused
P. syriaca (Syrian coney)	Hyracoidea	Fused	Fused
P. abyssinica (Abyssinian coney)	Hyracoidea	Fused	Fused
Dendrohyax dorsalis (Gold Coast tree-coney)	Hyracoidea	Fused	Syndesmosis
**Rattus norvegicus* (brown rat)	Rodentia	Synovial	Fused
**Mus musculus* (house mouse)	Rodentia	Synovial	Fused
Bathyergus maritimus (African sand-mole)	Rodentia	Fused	Fused
**Cavia cobaya* (guinea-pig)	Rodentia	Synovial	Fused
Jaculus jaculus (African jerboa)	Rodentia	Synovial	Fused
**Oryctolagus cuniculus* (rabbit)	Lagomorpha	Synovial	Fused
Lepus europaeus (hare)	Lagomorpha	Synovial	Fused
Priodontes gigas (giant armadillo)	Edentata	Fused	Fused
Orycteropus afer (aard-vark)	Tubulidentata	Fused	Syndesmosis
**Tarsius spectrum* (tarsier)	Primates	Synovial	Fused

It may be seen from Table 1 that within the same zoological order the tibio-fibular articulations may show quite different characteristics. For example, the fused upper and lower tibio-fibular joints in the African sand-mole, *Bathyergus maritimus*, appear to make this species unique in this respect in its own particular order. Among the Hyracoidea, a synostosis at the upper end and a syndesmosis at the lower are typical of tree-living varieties, while a synostosis at both ends is found among the fossorial coneys. On the other hand, similar tibio-fibular articulations are found in members of quite different orders; for example, a synovial joint at the upper end of the fibula with a synostosis at the lower is found among many Insectivora (e.g. the European mole, *Talpa europaea*), in most members of the Rodentia and Lagomorpha (e.g. the jerboa, *Jaculus jaculus*, and the common rabbit, *Oryctolagus cuniculus*), and within the Primates (the tarsier, *Tarsius spectrum*). These observations suggest that identical forms of tibio-fibular articulation in diverse mammalian types are the results of functional adaptations and are independent of taxonomic considerations (Fig. 11).

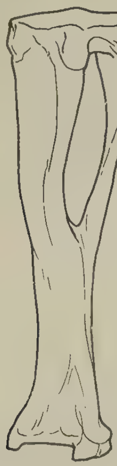

Fig. 1

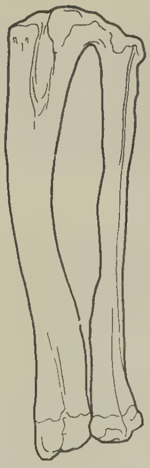

Fig. 2

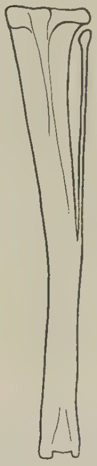

Fig. 3

Fig. 1. Cape golden mole (*Chrysochloris asiatica*). ×5.
Fig. 2. Grey seal (*Halichoerus grypus*). ×$\frac{1}{4}$.
Fig. 3. African jerboa (*Jaculus jaculus*). ×$1\frac{1}{4}$.

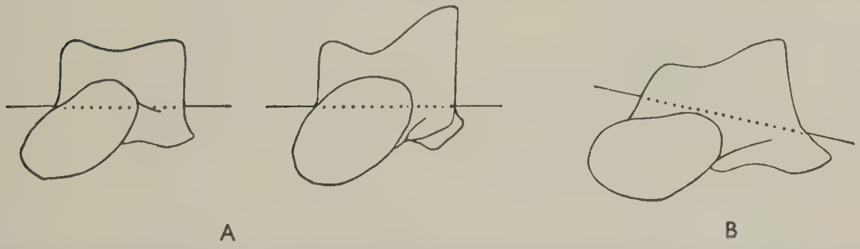

Fig. 4. Diagrams illustrating the relation of the axis at the ankle joint to the lateral articular surface of the talus. A, tali found in association with the immobile fibula—the axis may or may not be parallel to the upper surface; B, talus usually found in association with the mobile fibula.

(2) *The mobile fibula*, where both upper and lower tibio-fibular joints are synovial, is found in only two orders, the Carnivora and the Primates (Table 2).

Table 2. *The mobile fibula*

(Both the superior and the inferior tibio-fibular joints are synovial in all the species listed in the table. Where wet specimens have been examined they are indicated by an asterisk.)

Order: Primates

**Lemur catta* (ring-tailed lemur)
L. fulvus (brown lemur)
L. variegatus (ruffed lemur)
**Perodicticus potto* (common potto)
Cebus capucinus (capuchin)
Ateles frontatus (spider monkey)
**Macaca mulatta* (Rhesus monkey)
M. speciosa (stump-tailed macaque)
M. nemestrina (pig-tailed macaque)
Papio hamadryas (sacred baboon)
**P. cynocephalus* (yellow baboon)
P. anubis (anubis baboon)
P. porcarius (chacma baboon)
Mandrillus sphinx (mandrill)
Hylobates hoolock (gibbon, hoolock)
H. lar (gibbon, lar)
Simia satyrus (orang-utan)
**Pan troglodytes* (chimpanzee)
Gorilla gorilla (gorilla)

Order: Carnivora

**Felis domesticus* (domestic cat)
Panthera leo (lion)
**P. tigris* (tiger)
P. pardus (leopard)
Lynx lynx (lynx)
**Lutra lutra* (otter)
**Ailuropoda melanoleuca* (giant panda)
**Selenarctos tibetanus* (Himalayan black bear)
Ursus arctos (brown bear)
Thalarctos maritimus (polar bear)

In the twenty-nine species examined the upper articulation consisted of a large synovial joint, the lower of a short syndesmosis and, in addition, a large semilunar facet on the tibia covered by articular cartilage and in continuity with the ankle joint (Figs. 5 and 6). Rotatory mobility of this type of fibula was demonstrated by means of metal indicator pins driven into the shafts of the leg bones in six wet specimens (chimpanzee, baboon, lemur, bear, tiger and cat) stripped of their muscles with their ligaments left intact.

In chimpanzee, lemur and bear, the mobile phase of fibular movement occurs as the foot is passively dorsi-flexed from the neutral position. Complete separation of the bones is prevented by the tension in the posterior talo-fibular and transverse tibio-fibular ligaments, while the anterior talo-fibular and tibio-fibular ligaments are relaxed, allowing the fibula to hinge posteriorly. The movement of lateral rotation can be demonstrated after all the ligaments have been divided—provided the fibula is held in contact with the side of the talus—thus indicating that the main factor in producing the movements is the conformation of the lateral side of the talus.

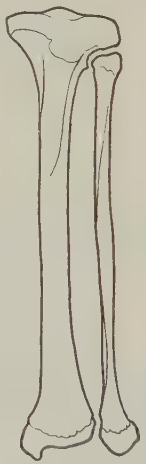

Fig. 5

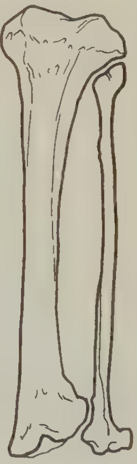

Fig. 6

Fig. 5. Orang-utan, *Simia satyrus*. $\times \frac{1}{4}$.

Fig. 6. Himalayan bear, *Selenarctos tibetanus*. $\times \frac{3}{10}$.

In the cats and the baboon, on the other hand, fibular rotation is limited to the plantar-flexion phase, the bone undergoing a medial rotation as the foot is plantar-flexed from the neutral position. In the cat, the anterior ligaments remain taut and the hinge around which the fibula rotates is therefore placed anteriorly.

The range of movement in all species is small and does not exceed 7 degrees, the greatest mobility occurring in the cat family. Other movements of the fibula have been observed but they appear to be of secondary importance to rotation.

In this group, the sides of the talus are often divergent; this is especially marked in the larger Primates and the Ursidae (Fig. 4B).

(3) *The intermediate fibula.* All the species so far mentioned have either an immovable joint at one or other end of the fibula, thus eliminating all rotatory movement, or mobile joints at both ends in which case rotation is clearly possible. There is, however, a further group in which rotational movements can theoretically occur. A synovial tibio-fibular joint is found superiorly, and a syndesmosis, commonly associated with a small synovial cavity, inferiorly. This group consists of a few members of several orders (Table 3), including a single representative of the Primates: the tree shrew, *Tupaia belangeri* (Fig. 7). In the Canidae, the syndesmosis is large, extending for at least one-third of the length of the shaft. Since by the study of the dry bones the possibility of rotatory mobility could not be excluded, metal indicator pins were driven into the leg bones of ligamentous preparations in two species, the domestic pig and the dog (three specimens). In neither species could any rotation be demonstrated on passive movements of the foot.

Table 3. *The intermediate fibula*

(All the species listed in the table have a synovial joint at the upper end and a syndesmosis at the lower end, often associated with a small synovial recess. Where wet specimens have been examined they are indicated by an asterisk.)

Species	Order
Crocuta crocuta (spotted hyaena)	Carnivora
*_Canis familiaris_ (domestic dog)	Carnivora
*_Vulpes vulpes_ (common fox)	Carnivora
Meles meles (badger)	Carnivora
Hylochoerus meinertzhageni (African forest hog)	Artiodactyla
Peccari tajacu (collared peccary)	Artiodactyla
*_Sus scrofa_ (domestic pig)	Artiodactyla
Hippopotamus amphibius (hippopotamus)	Artiodactyla
Rhinoceros unicornis (Indian rhinoceros)	Perissodactyla
Diceros simus (white rhinoceros)	Perissodactyla
Tapirus indicus (Malayan tapir)	Perissodactyla
T. bairdii (Baird's tapir)	Perissodactyla
Elephas maximus (Indian elephant)	Proboscidae
Sciurus vulgaris (red squirrel)	Rodentia
Castor fiber (beaver)	Rodentia
Myrmecophaga tridactyla (great anteater)	Edentata
Tupaia belangeri (tree-shrew)	Primates

It has been noted in some species with this type of tibio-fibular articulation that the joints tend to ossify in old age, e.g. hippopotamus and tapir.

Highly specialized types. The functional requirements of certain species result in a highly specialized hind-limb leading to radical changes in the relationship of the fibula to the tibia and tarsus, and in the form of the talus. Among the Chiroptera, the fruit bat, *Pteropus aegypticus,* shows an ankle joint with a ball-and-socket mechanism to permit adduction and abduction of the foot, and a greatly reduced fibula. Among the Edentata, the collared sloth, *Bradypus torquatus,* possesses a ball-and-socket articulation between the lower end of the fibula and the talus;

this specialization is presumably related to the unique mode of progression of this animal, the limbs being used to support the weight of the dependent trunk.

The Artiodactyla, with the exception of the few species referred to above, have lost the upper part of the fibula but have retained the lower end in the form of an os malleolare. These modifications may be correlated respectively with the absence of inversion and eversion of the foot rendering a large bony origin for the peronei redundant, and with the necessity for maintaining a stable hock joint. The talus is markedly trochleariform and the lateral side consists of a deeply concave articular facet for reception of the os malleolare. In two of the seventeen specimens examined, the cow, *Bos taurus*, and sheep, *Ovis aries* (Fig. 9), experiments were carried out to determine mobility of the os malleolare in wet specimens. The only movement that could be detected was a very small amount of splaying of the bone around an approximately horizontal hinge in full plantar-flexion. No axial rotation was demonstrated.

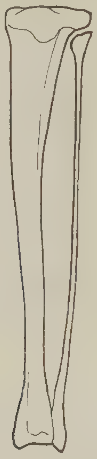

Fig. 7

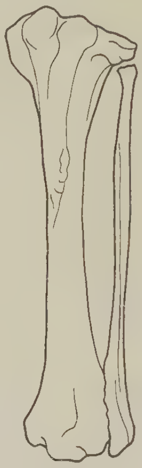

Fig. 8

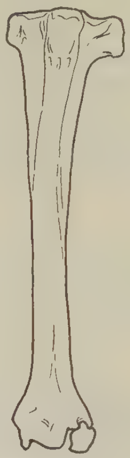

Fig. 9

Fig. 7. Tree shrew, *Tupaia belangeri*. ×1¼.
Fig. 8. Malayan tapir, *Tapirus indicus*. ×¼.
Fig. 9. Sheep, *Ovis aries*. ×¼.

In Equidae the lower end of the fibula is completely incorporated into the tibia and there is no true lateral malleolus. In these animals stability of the ankle joint is assured however by means of the closely interlocking nature of the bones, rendering the presence of a lateral buttress inessential.

DISCUSSION

Two types of tibio-fibular articulation have been found associated with the *immobile* type of fibula:

(*a*) A slender, flexible fibula with an upper synovial joint and a synostosis at the lower end, which in many species may be actually incorporated with the tibia. The functional significance of this type of fibula is obscure. Its extreme flexibility, however, suggests that distortion of the bone may occur in the initial phase of contraction of the attached muscles during the period when the inertia of the ankle joint and the tone of the antagonist muscles is still being overcome. Greater initial velocity results when joint movement is finally brought about by the fully contracted muscle. The action of the fore-finger and thumb in flicking a pellet is a familiar example of a similar mechanism, joint movement being held back until the agonists are fully contracted and the antagonists fully relaxed. This hypothesis receives support from the observation that this type of fibula is present in those creatures which are capable of sudden, rapid movements of jumping and bounding such as the hare, the jumping shrew, the jerboa and the tarsier. It is of interest that a similar tibio-fibular articulation is found in the saltatorial marsupial, the red kangaroo.

(*b*) A stout, inflexible fibula fused to the tibia at the upper end and united at the lower end by bone or by fibrous tissue is characteristic of the swimming or burrowing mammals, for example, the seal, the cape golden mole, the desman and the armadillo. This arrangement, which provides a rigid origin for muscles acting on a free foot, is requisite for swimming and burrowing activities where there is no distal fixed point of leverage such as is provided by the ground during terrestrial progression.

The *mobile fibula* is the less common type, and is virtually limited to cats and bears among the Carnivora and to the majority of Primates. The fibula articulates with the tibia superiorly by a large horizontally directed synovial joint and inferiorly by a synovial joint which is typically semilunar in shape; thus fibular rotation is theoretically possible throughout the range of ankle joint movement, although in practice it is usually confined to a certain phase. All these animals are either remarkably well adapted to progression, often at high speeds, over rocky and uneven surfaces (e.g. cats, bears, gorillas and baboons) or are wholly or partly arboreal (e.g. orang and chimpanzee). Both these modes of life are similar in that they not only demand a wide range of flexion and extension at the ankle but also tend to promote inversion and eversion strains in the same joint.

MacConaill (personal communication) has shown that a certain amount of axial (conjunct) rotation must occur at all hinge-joints in order to provide efficient lubrication. This effect is dependent upon the existence of an obliquely-placed axis of rotation. The evidence for this oblique axis at the ankle joint in this group of Primates and Carnivora is provided by the unequal curvatures of the medial and lateral sides of the talus, as has already been shown in man (Barnett & Napier, 1952). In these animals the lateral side of the talus is thus not perpendicular to the axis of rotation (Fig. 4). It necessarily follows that, as the talus is dorsi-flexed, the plane of the lateral side changes, in accordance with the principle that unless a rotating surface is perpendicular to the axis about which it is moving its plane cannot remain constant. The fibula must undergo a corresponding lateral rotation if the adjacent surfaces are to remain in contact. Since the fibula is thick and inflexible such rotation is possible only in the presence of movable joints at both upper and lower ends. In general, the degree of conjunct rotation in a hinge-joint is proportional to the functional range of movement. In this group of mammals the range of ankle movement is great (Young, 1950), and thus if contact between the talus and fibula is to be maintained—and this is essential in animals which are subject to constant inversion and eversion strains—the fibula must be adjustable. Fibular mobility is therefore essentially a mechanism to stabilize the ankle joint in animals whose way of life depends on speed and agility over an irregular terrain. In the cat family and the baboons, where the gait is digitigrade, the need for stability is greatest during plantar-flexion. It is mainly during this phase that fibular rotation occurs in these animals. On the other hand, in those primates and bears that exhibit a plantigrade type of gait the need for stability is greatest during dorsi-flexion. Experimental findings have confirmed that rotation of the fibula occurs during this phase.

In the larger Pongidae and the bears the sides of the talus are divergent; this is presumably related to a frequent assumption in these animals of an upright posture which necessitates a wide foot through which the body weight can be distributed.

It is probable that the os malleolare in the Artiodactyla likewise serves to stabilize the ankle joint against inversion and eversion strains, to which these animals are prone as a result of the cursorial characteristic of an elongation of the distal limb segment. A similar elongation is found in the Equidae; here the proximal portion of the fibula, articulating with the tibia at its upper end by means of a synovial joint, may be comparable in function to the slender fibula of saltatorial mammals. The movement permitted at the ankle is a hinge-like flexion and extension about an axis which is usually parallel to the ground and at right angles to the long axis of the limb. This is ideally adapted to a four-footed mode of progression, especially in cursorial mammals (Lull, 1904), where pure flexion and extension are required without the associated abduction and adduction which an axis not parallel to the ground would inevitably produce.

These conclusions concerning the functional significance of the mobile and immobile types of fibula receive interesting support from the consideration of two highly specialized species in which the combination of tibio-fibular articulations appears to be unique. The fibula of the cheetah, *Acinonyx jubatus*, is long and slender and is firmly united to the tibia by fibrous tissue in the middle third of its shaft. The superior and inferior joints are synovial (Fig. 10). The fibula is sufficiently flexible to allow movement at both the extremities of the bone in spite of the fixation of the mid-portion of the shaft. This unusual combination can be correlated with the remarkable speed of this animal which probably exceeds 65 miles/hr. over short distances (Howell, 1944). The inferior articulation is characteristic of those animals adapted for progression over uneven surfaces, while the upper joint in combination with the mid-shaft syndesmosis is similar to that found in the hare, jerboa and tarsier, all of which are capable of rapid jumping and bounding movements. The second animal of special interest is the coati, *Nasua nasua*, which possesses a slender fibula fused to the tibia at the upper end and articulating by a synovial joint at the lower, where rotatory movement is permitted by virtue of the flexibility of the fibula. The upper end is typical of swimming and burrowing animals, while the lower end again resembles that found among animals adapted for uneven surfaces. This combination can probably be explained by reference to its reputed ability to dig burrows in the ground and also to hunt in trees.

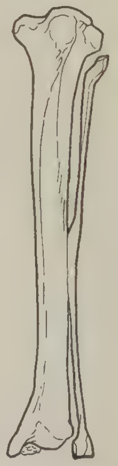

Fig. 10. Cheetah, *Acinonyx jubatus*. × $\frac{1}{4}$.

Representatives of the *intermediate type* of fibula are found in several mammalian orders. A small syndesmosis at the inferior articulation appears to be characteristic of those mammals which are not specialized in their mode of locomotion, that is to say those species showing no modification towards a cursorial, saltatorial, fossorial or aquatic mode of life, e.g. the great anteater, tapir and hippopotamus.

The fibular mobility of this group, in which the upper joint is synovial, clearly depends on the extent of the syndesmosis at the lower end. Although no mobility has been detected experimentally in the dog and the pig, in which the syndesmosis is relatively large, the possibility of some movement cannot be excluded in those animals in which the syndesmosis is short.

It is evident that within this group there are several species which exhibit some of the characteristics of the specialized groups. For example, the red fox, *Vulpes vulpes*, possesses an extensive tibio-fibular syndesmosis associated with a slender, flexible fibula which is functionally reminiscent of that found in the group capable of sudden, rapid limb movements. No rotation was detected experimentally in this species. On the other hand, the spotted hyaena, *Crocuta crocuta*, which shows a well-marked semilunar articular facet between the lower ends of the tibia and fibula in addition to a short syndesmosis, resembles in this respect those animals which are specially adapted for progression over uneven surfaces. Man shows a similar tendency—although the inferior tibio-fibular articulation frequently lacks a well-marked synovial recess, there is evidence that rotation of the fibula occurs (Bonnin, 1950).

It seems probable that an articulation resembling the intermediate type has given rise, by adaptive radiation, to all the diverse forms of tibio-fibular relationship found in mammals with specialized modes of locomotion (Fig. 11).

This view is in accordance with that of Carleton, who points out that in primitive vertebrates the tibia and fibula were not in contact at the lower end. It is likely, therefore, that before the development of a synovial joint inferiorly there must have been a phase where the two bones were united by fibrous tissue. This condition is illustrated in the present series by the intermediate type. Matthew (1937), on the other hand, maintains on palaeontological grounds that the type of articulation now found in large arboreal mammals represents the primitive placental form.

It is widely accepted that the common ancestor of all placental mammals was a small arboreal insectivore resembling in size and mode of life the modern tree-shrew, which in common with other small arboreal forms belongs to the *intermediate* group. It can be inferred that these mammals have retained the ancestral tibio-fibular relationship, which then, as now, was presumably determined by functional needs. These small arboreal mammals, which can progress along the curved surface of a branch as if it were flat, are in contrast to the large arboreal forms in which a readily adaptable foot is a prerequisite; as described above, such adaptability necessitates the presence of a mobile fibula. Increase in size, therefore, may have been largely responsible for the development of an inferior synovial joint. The somewhat unspecialized tibio-fibular articulation of man may indicate an early divergence from the primitive stock at a stage when his arboreal ancestor was still of small size.

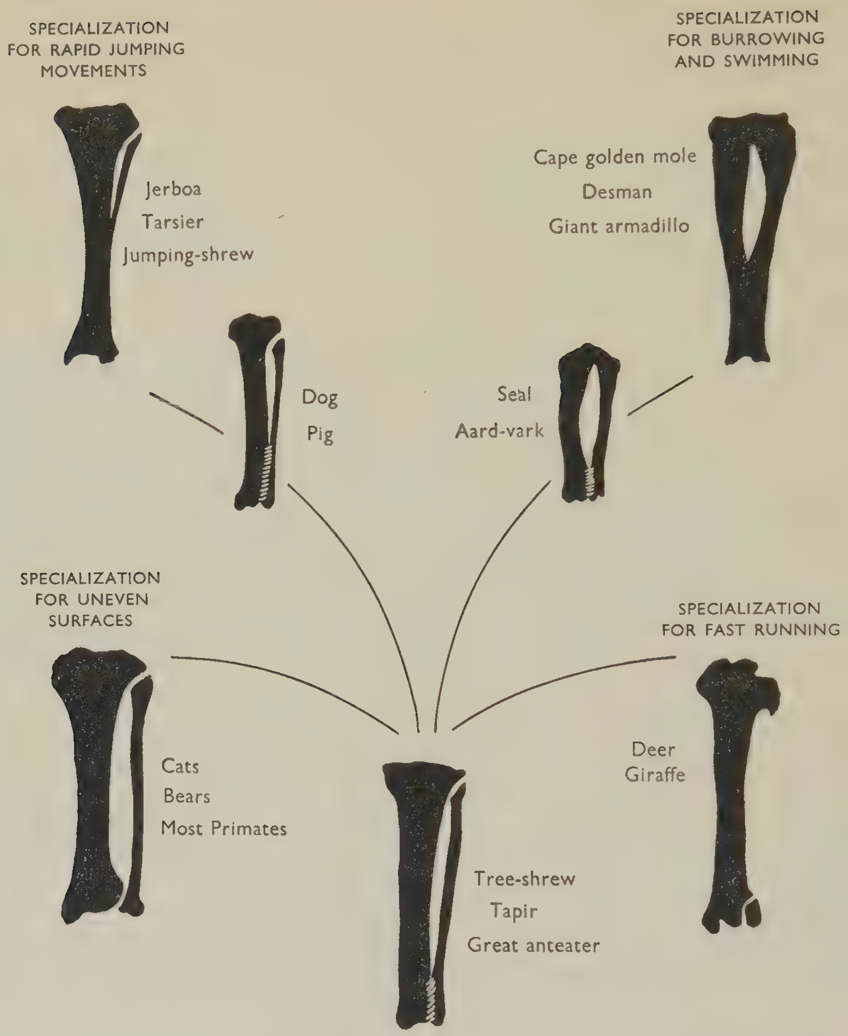
SPECIALIZATION FOR RAPID JUMPING MOVEMENTS
Jerboa
Tarsier
Jumping-shrew
SPECIALIZATION FOR BURROWING AND SWIMMING
Cape golden mole
Desman
Giant armadillo
Dog
Pig
Seal
Aard-vark
SPECIALIZATION FOR UNEVEN SURFACES
Cats
Bears
Most Primates
SPECIALIZATION FOR FAST RUNNING
Deer
Giraffe
Tree-shrew
Tapir
Great anteater

Fig. 11. Scheme illustrating an adaptive radiation from a primitive type of tibio-fibular articulation towards four important specializations, with examples from different orders.

SUMMARY

1. The rotatory mobility of the fibula has been studied in the hind-limb bones of ninety-two representative eutherian mammals. In addition, twenty-two wet specimens have been dissected, and in many of these the relative movements of the fibula and tibia have been directly observed by means of indicator pins driven into the bones after they have been stripped of all muscles. Where there is fibular mobility, the principal movement is a lateral rotation occurring as the foot is passively dorsi-flexed from the plantar-flexed position.

2. An attempt is made to explain the mechanisms underlying fibular mobility among the mammals.

3. On the basis of the anatomy of the tibio-fibular articulations it is possible to classify the majority of fibulae into three main types: the *immobile* fibula, fused to the tibia at one or both ends; the *mobile* fibula, articulating with the tibia by synovial joints at both ends; and the *intermediate* type, where the upper joint is synovial and the lower a syndesmosis.

A few mammals show specializations of the fibula which exclude them from a classification of this nature. These are briefly discussed.

4. The *immobile* fibula is always associated with a talus of which the lateral articular surface is perpendicular to the axis of rotation at the ankle joint. There are two main varieties:

(*a*) A thin, flexible fibula, fused or incorporated with the tibia at its lower end, and articulating with it at the upper end by means of a synovial joint. This variety is typical of limbs capable of sudden, rapid movements, as in saltatorial mammals.

(*b*) A stout, inflexible fibula, fused with the tibia at its upper end and united with it at its lower end by bone or fibrous tissue. This variety is characteristic of fossorial and aquatic mammals.

5. The *mobile* fibula is restricted to certain members of the Carnivora and the Primates, and is associated with the ability to adapt the foot to uneven surfaces. The lateral articular surface of the talus is seldom perpendicular to the axis of rotation at the ankle joint.

6. The *intermediate* type of fibula includes those approximating most closely to a primitive pattern, as well as others showing some degree of modification towards the types adapted for specialized modes of progression.

We are greatly indebted to Dr F. C. Fraser and Miss J. E. King of the Osteological section of the Zoology Department of the British Museum for the help they have given us throughout this investigation.

Our thanks are due also to Prof. F. Wood Jones and Prof. D. V. Davies for the material they have placed at our disposal.

We acknowledge with thanks the help of Miss F. Woolcombe who is responsible for the majority of the drawings and Mr A. L. Wooding and Mr J. B. Chanter for the photographic work.

REFERENCES

Barnett, C. H. & Napier, J. R. (1952). The axis of rotation at the ankle joint. *J. Anat., Lond.*, **86**, 1–9.

Bonnin, J. G. (1950). *Injuries to the Ankle*. London: Heinemann.

Carleton, A. (1941). A comparative study of the inferior tibio-fibular joint. *J. Anat., Lond.*, **76**, 45–55.

Howell, A. B. (1944). *Speed in Animals*. Chicago: University of Chicago Press.

Lull, R. S. (1904). Adaptations to aquatic, arboreal, fossorial and cursorial habits in mammals. IV. Cursorial adaptation. *Amer. Nat.* **38**, 1–11.

MacConaill, M. A. (1952). Personal communication.

Matthew, W. D. (1937). Paleocene faunas of the San Juan Basin, New Mexico. *Trans. Amer. phil. Soc.*, N.S., **30**.

Owen, R. (1866). Comparative Anatomy and Physiology of Vertebrates. II. London: Longmans, Green & Co.

Walmsley, T. (1918). The reduction of the mammalian fibula. *J. Anat., Lond.*, **52**, 326–331.

Young, J. Z. (1950). *The Life of Vertebrates*. London: Oxford University Press.

HISTOCHEMICAL STUDIES ON HUMAN SKIN AUTOGRAFTS

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INTRODUCTION

Although the histological changes occurring in grafted skin are well known (cf. Medawar, 1944; Gibson & Medawar, 1943), there is no information available about the metabolic changes which might be expected to occur. Knowledge of such changes is obviously of importance, since it may throw some light on the reasons for the usual failure of survival of skin homografts. The present work is concerned with the histochemical study of two epidermal components—glycogen and ribonucleic acid—in human skin autografts; the observations presented are intended to serve as a basis for a similar investigation of homografted skin.

MATERIAL AND METHODS

Skin was obtained from a male patient, T.M., aged 48 years, admitted to the Royal Infirmary, Glasgow, on 30 April 1951, suffering from burns of the right leg round the knee, on the medial aspect of the calf and on scattered areas of the hands and face. On two separate occasions pinch autografts were taken from the left thigh and grafted on to the burned area on the calf—on 14 May 1951 (series I) and on 21 May 1951 (series II). Biopsies were removed at intervals, each biopsy comprising one of the original pinch grafts and as little as possible of the surrounding tissue. In the following account the biopsies are identified by the series number I or II and by the number of days elapsing since the original grafting. Since no difference in behaviour of the two series can be detected, material from them has been pooled and treated as a single series of biopsies. In this way a more complete record has been obtained of changes occurring over a total period of 20 days.

The biopsies were fixed in ice-cold Rossman's fluid (absolute alcohol saturated with picric acid, 9 parts, neutral formalin, 1 part), dehydrated in absolute alcohol, cleared in benzene, embedded in paraffin wax and sectioned at 5μ.

Sections were stained by the following methods: (1) Harris's haematoxylin and eosin. (2) The Bauer-Feulgen method for glycogen using saliva-treated slides as controls. (3) 0·2% aqueous toluidine blue to demonstrate cytoplasmic basophilia: (*a*) after incubation for 3 hr. at 37° C. in McIlvaine's buffer at pH 7, containing 0·1 mg./ml. of crystalline ribonuclease; and (*b*) as a control, after incubation for 3 hr. in buffer alone.

The ribonuclease, highly purified and free from non-specific proteolytic activity, was generously provided by Prof. J. N. Davidson of the University of Glasgow.

OBSERVATIONS

(1) *Control skin removed at time of original grafting* (I–0)

Sections of a piece of skin removed at the time of grafting show the appearances expected in skin of moderate thickness—a stratum germinativum composed of small compactly arranged cells, a stratum granulosum of one or two cells thickness and a well-defined stratum corneum (Pl. 1, fig. 1). Mitotic activity is so low that, in many sections, careful search of the whole extent of the section (about 4 mm.) fails to reveal a single mitotic figure.

Sections stained with toluidine blue show moderate cytoplasmic basophilia in the basal one-half or more of the stratum germinativum and intense basophilia of the stratum corneum (Pl. 1, fig. 2). Sections stained with toluidine blue following digestion in a solution of ribonuclease show a marked reduction in the basophilic staining except in the stratum corneum (Pl. 1, fig. 3). It may be concluded that the cytoplasmic basophilia of the stratum germinativum is due to the presence of ribonucleic acid, which is specifically destroyed by ribonuclease, and that the basophilia of the stratum corneum is due to something other than ribonucleic acid.

The epidermal glycogen, as revealed by the Bauer-Feulgen staining procedure, is so sparsely distributed in these control sections of normal skin that it cannot be photographed satisfactorily. The small amount present is found in some of the more superficial cells of the stratum germinativum in the form of small intracellular granules.

(2) *Biopsy removed on 5th day after grafting* (II–5)

In describing the changes which occur in grafted skin, it is convenient to adopt the terminology of Gibson & Medawar (1943). The persisting epithelium of the original graft is termed 'graft roof' epithelium, that which grows out from the margin of the graft is called 'spread' epithelium.

The appearance of the graft roof epithelium is illustrated in Pl. 1, fig. 4, and the most obvious change is an increase in its thickness. This is due partly to cell proliferation—in a 4 mm. stretch of epidermis as many as twelve mitotic figures may be counted in a single section—and partly to cellular hypertrophy. This increase in cell size is particularly evident in the basal half of the stratum germinativum, where the cell boundaries are distinct. More superficially, cell boundaries are obscure, but the increase in mass of cytoplasm is evident from the wider spacing of the nuclei. The cytoplasm has an opaque, cloudy appearance. Comparison of figs. 1 and 4 in Pl. 1 shows that there has been an increase in nuclear and nucleolar size, and many nuclei have now two or more nucleoli. The stratum granulosum has disappeared, and keratinization involves two or three layers of surface cells, which still retain small condensed nuclei—a phenomenon usually referred to as parakeratosis. Here and there along the surface are sheets of dead epidermis, separated from the surviving epithelium by spaces containing a palely eosinophilic coagulum, in which are suspended red cells and polymorphonuclear and mononuclear leukocytes.

Sections of the graft roof epithelium stained with toluidine blue show intense cytoplasmic basophilia in the basal third of the stratum germinativum (Pl. 1, fig. 5). In the more superficial cells of the stratum germinativum the basophilia appears at first sight to be reduced as compared with that of normal skin. There is, however, a dilution factor, since the individual cells are much larger than in the normal epidermis, and each cell probably contains more, and certainly no less, ribonucleic acid than cells at a corresponding level in normal skin. That the basophilia of all except the cornified layers is in fact due to ribonucleic acid is illustrated by Pl. 1, fig. 6, which represents a section treated by ribonuclease prior to staining with toluidine blue.

The spread epithelium is thicker than that of the graft roof, and shows cellular and nuclear hypertrophy to an even greater degree. It is, moreover, extensively infiltrated with leukocytes. The cells stain palely with haematoxylin and with toluidine blue, and do not show the marked basophilia noted in the basal half of the graft roof epithelium.

Sections stained by the Bauer-Feulgen method show abundant glycogen in both graft roof (to right of figure) and spread epithelium, appearing in the photograph as black-stained material (Pl. 1, fig. 7). Glycogen is found only in the superficial half of the stratum germinativum. The black staining in the dead desquamated epithelium represents glycogen in leukocytes.

(3) *Biopsy removed on 7th day after grafting* (II–7)

At 7 days, the stratum granulosum has reappeared in the graft roof epithelium, indicating the return of the normal pattern of keratinization. It is thicker than normal, comprising four or five layers of cells. In the cells of the stratum germinativum, nuclei and nucleoli are still larger than normal, and the cytoplasm retains the homogeneous opaque appearance noted at 5 days. The spread epithelium is parakeratotic immediately adjacent to the graft roof, but is otherwise unchanged in appearance.

Sections stained with toluidine blue show in the graft roof a picture similar to that seen at 5 days—intense basophilia of the basal third of the epidermis, and somewhat paler staining of the more superficial cells. The spread epithelium differs in no way from that in the 5-day biopsy, showing little basophilia even in its basal layers.

Glycogen is still abundant in both graft roof and spread epithelium. It is confined, as before, to the superficial half of the stratum germinativum, and is still absent, in stainable quantities, from the basal and cornified layers.

(4) *Biopsy removed on 9th day after grafting* (I–9)

From the 9th day onwards the epithelium of the graft roof is characterized by hyperkeratosis and a thickened stratum granulosum. The cells of the superficial half of the stratum germinativum have lost the curious opaque homogeneous appearance seen at 5 and 7 days.

The pattern of basophilia shows little change. The spread epithelium, in which keratin formation is still minimal, remains relatively pale when stained with toluidine blue.

Glycogen attains its highest concentration. It is present throughout the thickness of the spread epithelium (Pl. 1, fig. 8), but in the graft roof is confined as before to the superficial half of the stratum germinativum (Pl. 1, fig. 9).

(5) *Biopsy removed on 12th day after grafting* (I–12)

At 12 days the graft roof epithelium is still thicker than normal, and is hyperkeratotic, with a many-layered stratum granulosum (Pl. 2, fig. 10). Cell outlines are now distinct throughout the thickness of the epidermis.

The only noteworthy change in sections stained with toluidine blue is in the spread epithelium, whose cells are now more basophilic, i.e. richer in ribonucleic acid. This change will be considered more fully in connection with the 16-day biopsy, which illustrates it very clearly.

Glycogen is abundantly present in the superficial half of the stratum germinativum in both the graft roof and spread epithelium.

(6) *Biopsy removed on 16th day after grafting* (II–16)

The changes which have been followed in the graft roof epithelium—hyperkeratosis and the restoration of the stratum granulosum—extend outwards into the spread epithelium. A section through the thick spread epithelium is illustrated by Pl. 2, fig. 11. The epidermis is hyperkeratotic and has a multi-layered stratum granulosum. In sections stained with toluidine blue (Pl. 2, fig. 12), the most intense cytoplasmic basophilia is seen in the basal half of the stratum germinativum, becoming less towards the surface. The basophilia is absent in ribonuclease-treated controls (Pl. 2, fig. 13). It is of interest that the keratohyalin granules of the stratum granulosum show negligible basophilia with toluidine blue.

The glycogen content of the epidermis is reduced, but still shows the characteristic localization (Pl. 3, fig. 14). Comparison of Pl. 2, figs. 11 and 12 and Pl. 3, fig. 14 shows clearly that glycogen is absent from the basal layers which are rich in ribonucleic acid; that it is present in the more superficial layers of the stratum germinativum; and that as keratohyalin granules accumulate within the cell, glycogen disappears.

(7) *Biopsy removed on 20th day after grafting* (II–20)

The biopsy removed on the 20th day was only a part of the original graft and does not appear to include any spread epithelium. The epidermis is still thickened, and hyperkeratotic but otherwise is almost normal (Pl. 3, fig. 15). The nuclei are now only slightly larger than normal (cf. Pl. 1 figs. 1 and 4 and Pl. 3, fig. 15). The intense basophilia noted in the basal half of the epidermis at earlier stages is reduced (Pl. 3, figs. 16, 17), and, finally, glycogen is once again reduced to a minimum, scarcely demonstrable by histological methods.

DISCUSSION

In the first few days after grafting, death and sloughing of localized epithelial areas occurs. This is probably due to the trauma of operation and to the relative ischaemia which must exist until the grafts are fully revascularized. At the 5th day the surviving epidermis is characterized by an increased rate of cell division, by an enlargement of the cells, nuclei and nucleoli, and by an increase in the ribonucleic acid content in its basal half. It is, in brief, a hyperplastic epithelium. The changes in nuclear and nucleolar size and the increase in cytoplasmic ribonucleic acid are of particular interest. From the work of Caspersson, Brachet and others it is now well-known that actively growing cells, and cells engaged in rapid synthesis of protein, show a large nucleolus and have a high content of cytoplasmic ribonucleic acid. The cells of the graft roof respond to grafting by hyperplasia and, later, by increased production of the protein keratin. One is not surprised, therefore, to find nucleolar enlargement and increased basophilia.

The other changes noted at the 5th day—the loss of clear cell outlines, the clouding of the cytoplasm and the parakeratosis—soon disappear. The granular layer has reappeared at the 7th day, indicating the restoration of the normal mechanism of keratin synthesis, and by the 9th day the other cellular abnormalities have disappeared. From the 7th to the 20th day, the graft roof epithelium is hyperkeratotic. During this hyperkeratotic phase there is a gradual though incomplete return to normal structure. Even at 20 days the epidermis is thicker than usual, but nuclear and nucleolar size is more normal. Mitotic activity is reduced and the intense basophilia of the cells of the basal half of the epidermis has faded.

The spread epithelium shows some interesting features. At 5 days the cells and their nuclei and nucleoli are very large, and the cytoplasm is pale. Parakeratosis is seen at 12 days. At 16 days the epithelium is hyperkeratotic, and the cells of the basal half show intense basophilia. The relative absence of basophilia at earlier stages is possibly partly a dilution effect, for the cells are very much enlarged. But there appears to be an absolute reduction in the ribonucleic acid content of the cells, the significance of which is obscure. As the spread epithelium ‘matures’—i.e. as cell size is restored to normal and the granular layer reappears—there is a re-accumulation of cytoplasmic ribonucleic acid.

Epidermal glycogen

In the control sections of normal skin, glycogen is present in minimal quantities. At 5 days it is abundant in both graft roof and spread epithelium and remains so until some time between the 12th and 16th days, when it is reduced. At 20 days it is present in little more than normal amounts. It is found in the superficial part of the stratum germinativum and is never present in stainable quantities in the basal and cornified layers of the graft roof. It shows a similar distribution in the spread epithelium except at the 9th day when it is abundant throughout the entire thickness.

It is difficult to assess the significance of these changes, particularly because little is known of the carbohydrate metabolism of normal skin. Cornbleet (1940) has shown that, whereas the glucose content of normal skin varies with that of the blood, the glycogen content is relatively constant. This has led him to conclude that ‘cutaneous glycogen does not exist merely to furnish locally needed glucose’.

Dickens & Weil-Malherbe (1943) and Berenblum, Chain & Heatley (1940) have shown that normal skin ferments glucose to lactic acid in the absence of oxygen (anaerobic glycolysis), and that the fermentative breakdown persists, although to a lesser extent, even in the presence of oxygen (aerobic glycolysis). That the cells should continue to use a glycolytic or fermentative mechanism even in the presence of oxygen is thought to indicate a defect in the oxidative mechanisms (cf. Barron, 1952). It is not known whether glycogen or glucose provides the usual starting-point of carbohydrate breakdown, nor how far skin depends upon aerobic oxidative mechanisms and how far upon fermentative ones. Some light is thrown on the second problem by the tissue culture experiments of Medawar (1947), who showed that skin will survive for as long as a week in the total absence of oxygen, and that this survival presumably involves fermentative breakdown of sugar, since it is inhibited by iodoacetate. On the other hand, cell growth and cell movement occur in culture only in the presence of oxygen.

From a theoretical standpoint there is a number of possible interpretations of the increase of glycogen in grafted epidermis.

(1) The increase of glycogen content might result from a more sluggish metabolism, with reduced carbohydrate utilization. The histological signs of increased cellular activity already discussed make this hypothesis unlikely.

(2) Glycogen accumulation might be regarded as a 'degenerative' phenomenon. 'Glycogenic degeneration' is common in rapidly growing and embryonal tumours (cf. Ewing, 1942). Glycogen accumulates in the superficial cells of the rodent vagina at pro-oestrus (Tribby, 1943) and in the cells of deciduomal plaques in the monkey (Rossman, 1940), and in each case the suggestion is offered that the reaction is degenerative and consequent upon a reduced blood supply. In grafted skin, however, the glycogen accumulation appears to outlive the period of poor vascularity.

(3) The accumulation of glycogen may represent a cellular adaptation to conditions of reduced oxygen tension. In seeking for a single common factor which might explain the accumulation of glycogen in a wide variety of situations, Dempsey & Wislocki (1944) have noted an inverse relationship between glycogen deposition and the activity of aerobic oxidative mechanisms. Their thesis is briefly this: that in the relative absence of oxygen (or inadequacy of aerobic oxidative mechanisms), cells will adopt anaerobic fermentative mechanisms, which are far less efficient, and which therefore require the presence of larger quantities of substrate—'an intense anaerobic metabolism demands a large accumulation of glycogen for the purpose of maintaining the fermentative processes'.

In applying this hypothesis to the present problem, it is interesting to recall that foetal epidermis is rich in glycogen. While nothing is known of the enzymic equipment of foetal epidermal cells for aerobic oxidative processes, it is reasonable to assume that foetal epidermis shares with other foetal tissues the relative anoxia of intra-uterine life. Glycogen has also been reported by Bradfield (1951) as abundant in the epithelium growing across healing experimental skin wounds in guinea-pigs. Bradfield's view is that the newly formed epidermis is thick and avascular, and its cells, poorly supplied with glucose and oxygen, are therefore forced to adopt anaerobic mechanisms of breakdown of sugar. Since the anaerobic mechanism is far less efficient than that of complete oxidative breakdown, 'for a given amount of protein synthesis, the epidermal cells will therefore require abnormally large quantities of glucose; but here again the absence of blood vessels must pose serious difficulties...hence it is not surprising to find that the cells store large reserves of glycogen...' (Bradfield, 1951).

Quite apart from its teleological flavour, Bradfield's view, which has much in common with that of Dempsey & Wislocki (1944), seems to overlook an important problem, namely, what is the source of the glycogen which accumulates? If, as seems likely, it is glucose from the blood, why is this glucose not utilized directly as an energy source? If its cells are so 'poorly supplied with glucose', how do they manage to store such large quantities of glycogen?

It seems, then, that none of the possible explanations of the accumulation of glycogen is entirely satisfactory, and the phenomenon can merely be recorded as one of the characteristics of grafted epidermis. From a purposive viewpoint, the significance of the accumulation is less obscure, particularly when the changes in glycogen and ribonucleic acid are correlated. The most intense cytoplasmic basophilia—i.e. the highest concentration of ribonucleic acid—is found in the basal layers of the epidermis. As the cells move towards the surface it seems likely that the ribonucleic acid participates in the synthesis of keratin. Glycogen is found in this zone of protein synthesis, between the basal and granular layers, and it is reasonable to assume that its breakdown provides some of the energy for this synthetic activity. Glycogen has certainly disappeared—presumably because it has been utilized—by the time the cells show keratohyalin granules.

SUMMARY

1. Glycogen and ribonucleic acid have been studied histochemically in biopsies of pinch autografts of human skin, at intervals of up to 20 days after grafting.

2. Epidermis responds to grafting by cellular hypertrophy and hyperplasia, followed by hyperkeratosis, with a gradual restoration of normal skin structure. Correlated with the hyperplastic changes there is hypertrophy of the cellular apparatus concerned with protein synthesis—the nucleolus and the cytoplasmic ribonucleic acid.

3. Control sections show minimal quantities of epidermal glycogen. Glycogen is abundant in biopsies removed at 5, 7, 9 and 12 days after grafting. It is found in the graft roof epithelium and in the epithelium spreading from the margins of the graft. It is present in the cells of the superficial half of the stratum germinativum.

4. The significance of the glycogen accumulation is discussed.

The skin was obtained through the kind collaboration of Mr Ian Macgregor, of the Unit of Plastic Surgery in Glasgow, under the charge of Mr J. S. Tough.

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REFERENCES

Barron, E. S. G. (1952). The oxidative pathways of carbohydrate metabolism. In *Modern trends in Physiology and Biochemistry*, pp. 471–507. New York: Acad. Press. Inc.

Berenblum, I., Chain, F. & Heatley, N. G. (1940). The metabolism of normal and neoplastic skin epithelium. *Amer. J. Cancer*, **38**, 367–371.

Bradfield, J. R. G. (1951). Glycogen of vertebrate epidermis. *Nature, Lond.*, **167**, 40–41.

Cornbleet, T. (1940). Cutaneous carbohydrates. I. The normal skin. *Arch. Derm. Syph.* **41**, 193–213.

Dempsey, E. W. & Wislocki, G. B. (1944). Observations on some histochemical reactions in the human placenta, with special reference to the significance of the lipoids, glycogen and iron. *Endocrinology*, **35**, 409–429.

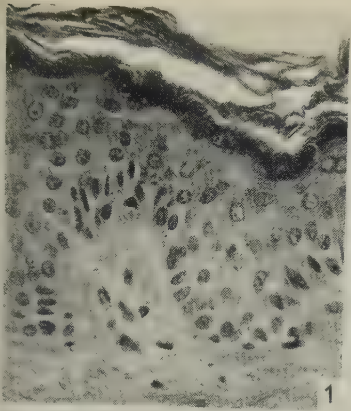
Dickens, F. & Weil-Malherbe, H. (1943). The metabolism of normal and tumor tissue. A comparison of the metabolism of tumors of liver and skin with that of the tissue of origin. *Cancer Res.* **3**, 73–87.

Ewing, J. (1942). *Neoplastic Diseases*. Philadelphia and London: W. B. Saunders and Co.

Gibson, T. & Medawar, P. B. (1943). The fate of skin homografts in man. *J. Anat., Lond.*, **77**, 299–310.

Medawar, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176–199.

Plate 1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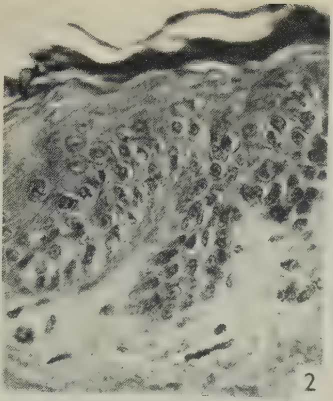
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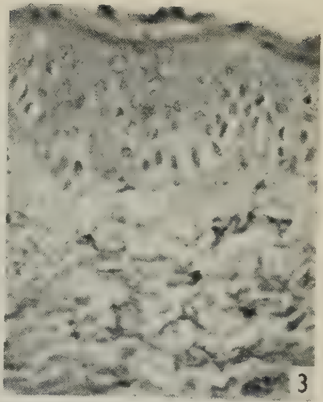
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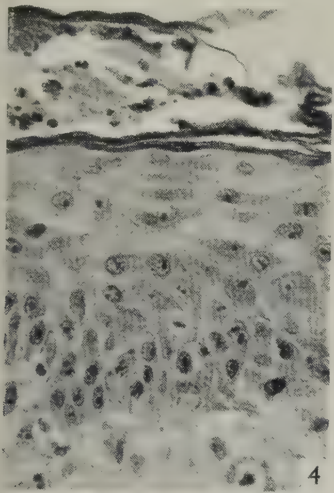
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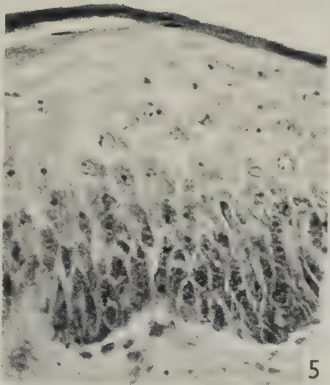
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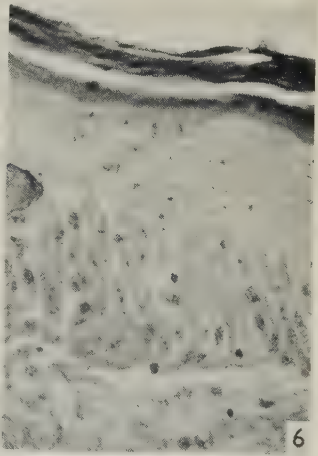
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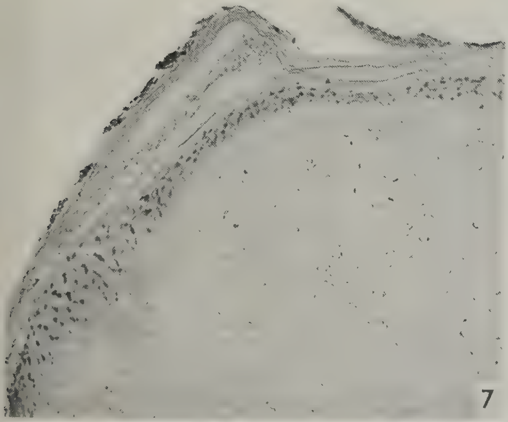
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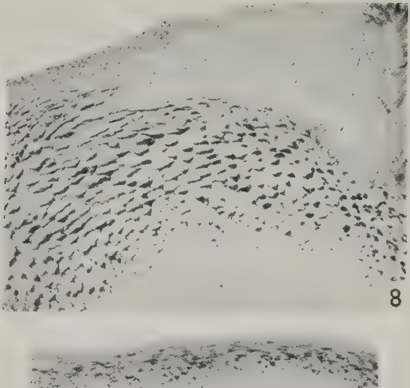
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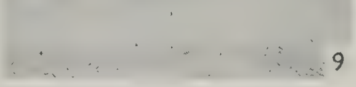
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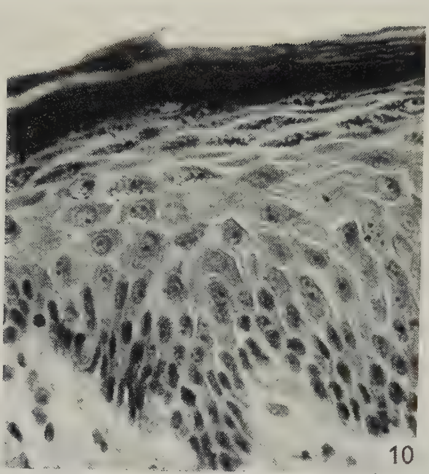
SCOTHORNE AND SCOTHORNE—HISTOCHEMICAL STUDIES ON HUMAN SKIN AUTOGRAFTS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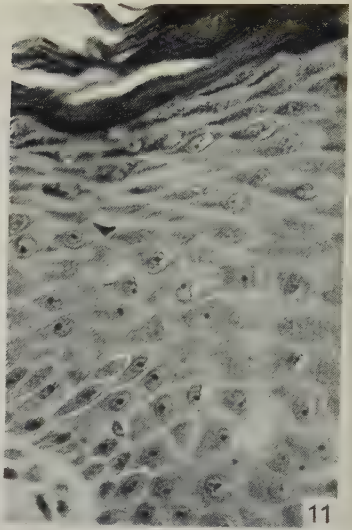
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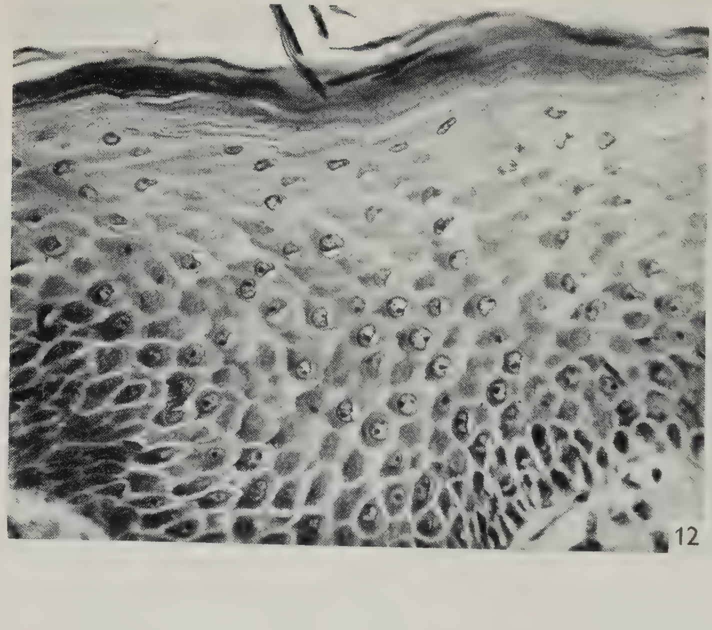
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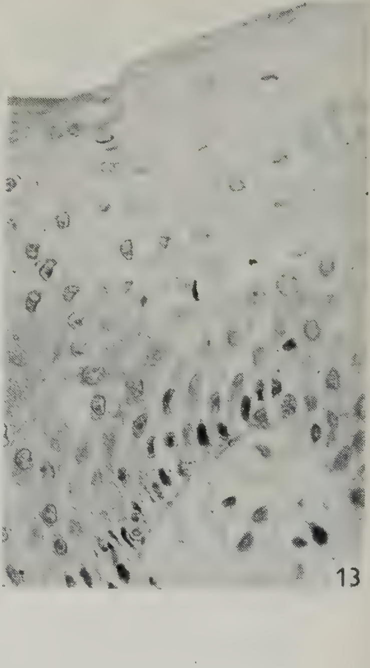
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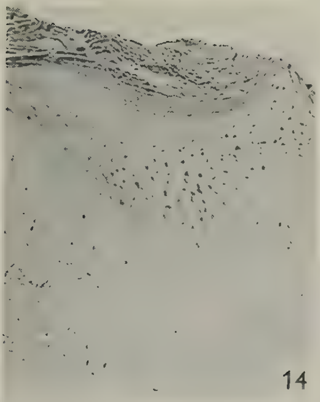
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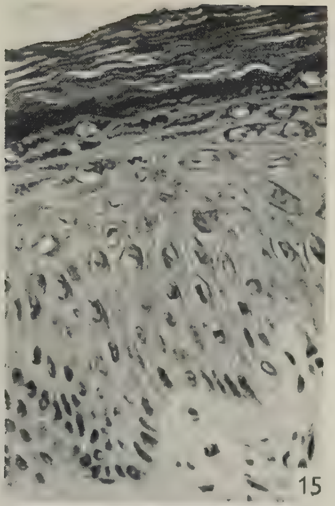
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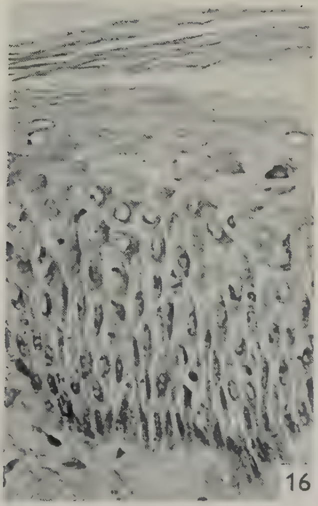
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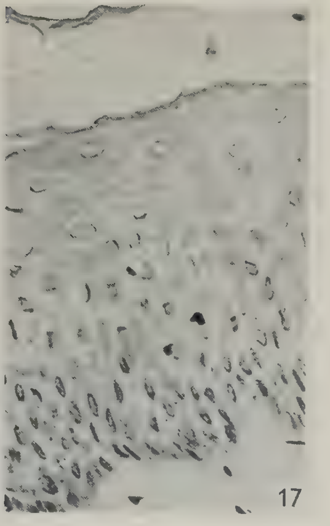
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SCOTHORNE AND SCOTHORNE—HISTOCHEMICAL STUDIES OF HUMAN SKIN AUTOGRAFTS

Medawar, P. B. (1947). The behaviour of mammalian skin under strictly anaerobic conditions. *Quart. J. micr. Sci.* 88, 27–37.

Rossman, I. (1940). The deciduomal reaction in the rhesus monkey (*Macaca mulatta*). I. The epithelial proliferation. *Amer. J. Anat.* 66, 277–365.

Tribby, C. L. (1943). The intracellular lipin, mucoid, and glycogen of the vaginal epithelium of the guinea pig. *Anat. Rec.* 86, 425–451.

EXPLANATION OF PLATES

Plate 1

Fig. 1. Control section of skin removed at time of grafting. (I–0.) Haematoxylin and eosin. × 350.

Fig. 2. Control section. (I–0.) Toluidine blue. × 350.

Fig. 3. Control section. (I–0.) Toluidine blue following ribonuclease digestion. × 350.

Fig. 4. Biopsy 5 days after grafting (II–5). Haematoxylin and eosin. × 350.

Fig. 5. Five-day biopsy. (II–5.) Toluidine blue. × 350.

Fig. 6. Five-day biopsy. (II–5.) Toluidine blue following ribonuclease digestion. × 350.

Fig. 7. Five-day biopsy. (II–5.) Bauer-Feulgen method for glycogen. No counterstain. Graft roof epithelium on right of photograph, spread epithelium on left. × 75.

Fig. 8. Nine-day biopsy. (I–9.) Shows glycogen throughout thickness of spread epithelium. × 75.

Fig. 9. Nine-day biopsy. (I–9.) Glycogen in superficial half of stratum germinativum of graft roof epithelium. × 75.

Plate 2

Fig. 10. Twelve-day biopsy. (I–12.) Graft roof epithelium. Haematoxylin and eosin. × 350.

Fig. 11. Sixteen-day biopsy. (II–16.) Spread epithelium. Haematoxylin and eosin. × 350.

Fig. 12. Sixteen-day biopsy. (II–16.) Toluidine blue. × 350.

Fig. 13. Sixteen-day biopsy. (II–16.) Toluidine blue following ribonuclease digestion. × 350.

Plate 3

Fig. 14. Sixteen-day biopsy. (II–16.) Bauer-Feulgen stain for glycogen. × 75.

Fig. 15. Twenty-day biopsy. (II–20.) Graft roof epithelium. Haematoxylin and eosin. × 350.

Fig. 16. Twenty-day biopsy. (II–20.) Toluidine blue. × 350.

Fig. 17. Twenty-day biopsy. (II–20.) Toluidine blue following ribonuclease digestion. × 350.

THE EPINEURAL SHEATH OF A NERVE AS A BARRIER TO THE DIFFUSION OF PHOSPHATE IONS

By G. CAUSEY and ELIZABETH PALMER

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It was shown by Feng & Gerard (1930) that the connective tissue sheath of a nerve acts as a diffusion barrier. They recorded the action potential from nerves with and without sheaths after soaking in different solutions, and observed the time that elapsed before conduction was blocked. Lorente de Nó (1947) adopted a similar procedure, but measured the time required for the beginning of depolarization. He concluded that the 'penetration of substances across the connective tissue sheath takes place with great rapidity' (Lorente de Nó, 1950) and, further, in the same paper, that 'the epineurium is not a significant diffusion barrier'. Feng & Liu (1949) re-examined the problem and were able to confirm the original findings of Feng & Gerard, and their conclusions that 'the connective tissue sheath of amphibian sciatic nerve is an effective diffusion barrier' is now widely accepted (Hodgkin, 1949; Rashbass & Rushton, 1949; Huxley & Stämpfli, 1951; Schoepfle, 1950). The difference of opinion shown in the papers of Feng and Lorente de Nó may be due to a lack of investigation into the identity of the tissues removed by different investigators.

In the papers mentioned above there is no illustration of a nerve trunk after it has been stripped of its 'connective tissue sheath'. In fact, the only histological illustration is of a nerve with its sheath in the paper by Lorente de Nó (1950) in which the tissue sheath labelled epineurium appears to correspond to the perineurium of Key & Retzius (1876). As there seems to be no clear statement of the morphology of this 'connective tissue sheath' in the literature on this subject, a histological examination has been undertaken to see what part of the connective tissue of a nerve is, in fact, removed by a standard stripping method. With this standard stripping method an investigation has been carried out on the effect of the sheath on the transfer of phosphate ions using the isotope ^{32}P.

NOMENCLATURE

The naming of the different layers of connective tissue between nerve fibres and between bundles of nerve fibres is quite consistent in the descriptions of Ranvier (1878) and Key & Retzius (1876), although there has been some divergence in the use of their terminology in the subsequent literature. The fine connective tissue between the nerve fibres is called by Key & Retzius the endoneurium, and this corresponds to the intrafascicular connective tissue of Ranvier. The condensation of the above tissue, which is seen around nerve bundles (see Pl. 1, fig. 1), is called the perineurium by Key & Retzius and *gaîne lamelleuse* by Ranvier. Outside the perineurium is the loose connective tissue of the epineurium of Key & Retzius, which Ranvier calls the interfascicular connective tissue in multifascicular nerve trunks.

In this paper we have adopted Key & Retzius's terminology. The terminology of the sheaths of individual nerve fibres still shows some confusion (see Young, 1942), but does not affect this discussion.

METHOD

The experiments reported here are concerned mainly with the nerves of the hind-limb of rabbits. The sciatic nerve of the frog has also been examined. The peroneal, tibial and sural nerves of rabbits were removed under nembutal and ether anaesthesia and put in mammalian Ringer solution. All the observations were made *in vitro*.

The method used to remove the sheath was as follows: a length of nerve was placed on a slide with sufficient Ringer solution to prevent drying. Under a dissecting microscope the nerve fibres could then be clearly seen as a firm opaque bundle in the loose connective tissue. At the cut end of the nerve the connective tissue tended to retract a little from the nerve bundle, and this effect was enhanced by gentle teasing of the cut end. The end of the nerve was then seized with fine forceps and the connective tissue transfixed with a dissection needle. If small strands of connective tissue sheath were caught in the forceps these strands had to be cut, or a new hold on the nerve trunk taken before the sheath would slip off the nerve. Gentle traction on the connective tissue made it slip off the nerve bundle very easily. The unbranched length of the sural nerve from the thigh of the rabbit could be stripped very easily, and small vessels running into the nerve bundle from the sheath presented no obstacle. If, however, the nerve was branching, as in the distal parts of the tibial and peroneal nerves, then the stripping was most successful when done in a proximo-distal direction in relation to the original orientation in the animal. When stripped in the opposite direction the sheath was held up at the site of the branches.

Stripped and unstripped lengths of the same nerve were fixed in Flemming's solution for 24 hr. in wax-sealed bottles. After dehydration the nerves were embedded in wax and cut into transverse or longitudinal sections. The sections were stained either by Weigert's method for myelin and counterstained with light green, or by triple azan staining.

For the determination of phosphate transfer the lengths of nerve were firmly tied at both ends with silk and suspended in a vessel containing 200 ml. of Ringer's solution with about 25μc. of ^{32}P. The counting rate per ml. of this solution was about 200,000 counts/min./ml. Experiments with the tied ends of the nerve within or out of the solution showed no consistent difference in the rate of take up so long as the ends were firmly tied. The temperature of the bath was maintained between 35 and 39° C. on an electrically heated platform, and oxygen was bubbled in a steady stream through the solution, producing oxygenation and agitation.

Unstripped nerves, stripped nerves and sheaths were treated in sets in the same solution. After treatment the solution was again counted and had lost less than 5 % of its original counting rate.

The treated specimens were washed in a large volume (*c.* 700 ml.) of non-radioactive Ringer's solution, which was circulated and oxygenated by a small lift pump worked by the oxygen inflow. Each specimen was divided into two unequal parts, lightly blotted and weighed in covered weighing bottles. In some experiments the washing procedure was omitted.

The larger of the two parts of each specimen was ashed in 1 ml. of concentrated acid (equal parts nitric and sulphuric). The solution was poured into a test-tube and the ashing flask washed out with two lots of 2 ml. of distilled water. The solution was then brought to neutrality with caustic soda against phenolphthalein and the volume made up to 10 ml. with distilled water. These solutions were then counted on a Geiger-Müller counter using the fluid counter designed by Veall (1948). Counting rates were from 10 to 30 times background; a minimum of 2500 counts was made on each specimen and corrected for background and decay.

The smaller part was ashed in 1 ml. of perchloric acid, and the total phosphorus estimated by the method of Berenblum & Chain (1938). The intensity of the colour of the coerulomolybdate complex being measured in a Spekker photometer, calibrated with known strengths of potassium phosphate.

The radioactivity of any specimen was then expressed as the corrected counts per minute for 100 mg. wet weight divided by the total phosphorus in μg. per 100 mg. wet weight.

RESULTS

(*a*) *Histological*

Pieces of 3–4 cm. length of the tibial, peroneal and sural nerves of the rabbit and sciatic trunk of the frog were taken, and divided into two equal parts. One piece was fixed without stripping and the other piece was stripped according to the description given above. Proximal and distal halves were stripped at random. As Feng & Gerard (1930) stated, there is a distinct plane of cleavage, and once this plane has been found at the cut end of a nerve, the two parts of the nerve, connective tissue sheath and nerve trunk, separate with very little traction. In our hands the nerves of the rabbit showed this property even more clearly than frog nerves. In the sural nerve of the rabbit, of which lengths of 5 or 6 cm. can be obtained free of branches, it is possible to remove the whole length of the sheath in one continuous piece. Before the sheath is finally removed from the distal end of the nerve, it can, if desired, be drawn back again over the nerve trunk.

Pl. 1, figs. 2 and 3, show transverse sections, of the sciatic nerve of the frog, the piece shown in fig. 2 being unstripped and in fig. 3 stripped. Pl. 1, figs. 4 and 5, are similar preparations from the peroneal nerve of a rabbit. In the preparations here illustrated, and in the many others that we have examined, it is clear that the 'connective tissue sheath' is synonymous with the epineurium as defined in the section on nomenclature and that the perineurium remains after stripping, closely investing the nerve.

In Pl. 1, fig. 6, a portion of the periphery of a stripped sural nerve is shown at higher magnification; the lamination of the perineurium and the continuity of its inner lamellae with the endoneurium is shown. This continuity of the perineurium and endoneurium explains the results obtained when the perineurium was deliberately dissected off the nerve trunk. The perineurium does not strip off the nerve. It had to be dissected off bit by bit and this procedure resulted in the disruption of the compact nerve bundle and produced a leash of separate nerve fibres.

(*b*) *Exchange of* ^{32}P

If the epineurium is a barrier to the entry of phosphate ions into the nerve proper then on soaking a nerve in a solution containing phosphate ions labelled with ^{32}P there are three changes to be expected:

(1) If two pieces of nerve are put in the solution, one with the epineurium in place and the other without its epineurium and if after soaking the epineurium is removed from the first piece and the two now similar pieces of nerve are assayed for their content of radioactive phosphorus relative to the total phosphorus, then the piece which was soaked without its epineurium should contain relatively more ^{32}P than the piece which retained its epineurium during soaking.

(2) The epineurium itself may accumulate the ^{32}P and will therefore have a high concentration of ^{32}P.

(3) It may simply bar the diffusion of the phosphate ion and have a low ^{32}P content throughout.

Table 1. *The specific activity* $\frac{\textit{(counts/100 mg./min.)}}{\text{P}/100\textit{ mg.}}$ *of nerve sheath and sheathless nerve when soaked in a solution containing* ^{32}P *before stripping off the sheath, and after stripping off the sheath*

	Soaked before stripping		Stripped before soaking	
	Nerve (1)	Sheath (2)	Nerve (3)	Sheath (4)
Peroneal	3	133	54	1108
	18	675	98	170
	2	375	188	1015
	—	—	73	380
	2	538	34	324
Tibial	4	821	125	270
	6	1002	27	255
	12	501	106	812
	4	133	94	312
	2	310	49	335
	5·9 ± 1·8	499 ± 99·2	84·8 ± 15·5	498 ± 108·5

The proportion of radioactive phosphorus to total phosphorus was therefore estimated in the epineurium and in the nerve bundle in pieces of nerve from which the epineurium had been stripped before the specimens were put in the bath. A similar estimation was made in the epineurium and nerve bundle soaked in the same bath, the epineurium being removed from the nerve after soaking. These effects were examined in both the tibial and peroneal nerves of the rabbit, and the results obtained are presented in Table 1.

It appears that the specific activity of the nervous tissue, endoneurium and perineurium, is very low in those specimens which were obtained by removing the epineurium after soaking (col. 1). The specific activity is about one-fourteenth of similar pieces of nerve soaked in the same solution in which the epineurium had been removed before soaking (col. 3).

If the nerve had taken up more water when soaked after removal of the epineurium, there is a possibility that the total phosphorus per unit weight would be appreciably diminished and the specific activity thereby increased, but, in fact, there was no significant difference between the phosphorus content per unit weight of the two types of specimen ($483\,\mu g. \pm 39$ and $539\,\mu g. \pm 30$ respectively). The specific activity of the epineurium was high in all cases (cols. 2 and 4). There was no difference according to whether the epineurium was on the nerve or removed from the nerve when it was soaked.

It seemed possible that this very high specific activity of the epineurium was due to failure to remove the actual radioactive solution from the surface of the nerve. This probability was, however, excluded by the long period of washing in circulating ^{32}P free solution which was described above.

These experiments, therefore, show that the hypotheses (1) and (2) above are in agreement with the observed behaviour of the specimens.

DISCUSSION

As Feng & Gerard (1930) have shown, there is a plane of cleavage along which the 'sheath' of a nerve can be easily removed. According to our histological preparations this sheath corresponds to the epineurium. This plane is very distinct in mammalian nerves and may either be between mesodermal elements or, if one accepts Masson's (1942) hypothesis of the development of the connective tissue of peripheral nerves, at the junction between mesodermal elements and those developed from the lemmoblasts.

Huxley & Stämpfli (1951) have attributed the barrier effect of the sheath of nerve to the perineurium. It is possible that the accumulation of ^{32}P in the epineurium of our specimens may be due to a failure to pass through the perineurium. However, if this were the case two effects should follow: (1) with the perineurium still in place on the stripped nerve the uptake of ^{32}P by the nerve should not be fourteen times that of the nerve with perineurium and epineurium intact, and (2) if the phosphate ion diffuses easily through the epineurium but is held up by the perineurium, then prolonged washing, such as was used, should again cause the phosphate to diffuse out of the epineurium. Mullins (1950) states that removal of the (sheath) increases the uptake of ^{32}P twofold in frog nerves.

The criticism by Lorente de Nó of the theoretical argument underlying the experiments of Feng & Gerard (1930) and Feng & Liu (1949) is based on their use of the time interval required for a given solute in the solution to produce total block of conduction, which represents the sum of the time required for such a substance to reach the nerve fibre and then to act upon the least sensitive fibres. The use of comparisons of the effect of a given substance on intact nerve and nerves with the epineurium removed or split, is criticized on the ground that such interference with the nerve will basically alter its ability to conduct (Lorente de Nó, 1950, p. 198).

By using ^{32}P to follow the uptake of an ion the whole discussion of effect on function is avoided, and the simple physical properties of the nerve and its sheaths in a non-biological milieu can be studied. It is clear that in the living animal the milieu of the individual nerve fibre is not necessarily that of the outside of the epineurium. The blood vessels pass from the epineurium into the endoneurium and are there distributed in a longitudinal anastomosis along the nerve tract (Adams, 1942).

On the basis of our experiments we have come to the conclusion that, *in vitro*, it is the epineurium that acts as a barrier and has a delaying effect on the diffusion of phosphate ions into the nerve fibres and endoneurium.

SUMMARY

1. A histological investigation of the 'connective tissue sheath' of nerves has shown it to be identical with the epineurium of Key & Retzius. When the epineurium was stripped off the nerve trunk the perineurium was left attached to the nerve bundle.

2. The isotope ^{32}P was used to investigate the transfer of phosphate ions in nerves with and without their epineural sheath. It was found that:

(*a*) The specific activity of the nerve fibres was increased fourteen times by the removal of the epineurium before soaking in radioactive solutions.

(*b*) The specific activity of the epineurium was very high compared with the rest of the nerve trunk.

3. It is concluded that the epineurium is a barrier to the diffusion of phosphate ions *in vitro*.

We wish to thank Prof. J. Z. Young, F.R.S., for his interest throughout this work, and Mr J. E. Marner and Mr B. Whitton for technical assistance.

REFERENCES

Adams, W. E. (1942). The blood supply of nerves. *J. Anat., Lond.*, **76**, 323–341.

Berenblum, I. & Chain, E. (1938). An improved method for the colorimetric determination of phosphate. *Biochem. J.* **32**, 295–298.

Feng, T. P. & Gerard, R. W. (1930). Mechanism of nerve asphyxiation: with a note on the nerve sheath as a diffusion barrier. *Proc. Soc. exp. Biol., N.Y.*, **27**, 1073–1076.

Feng, T. P. & Liu, Y. M. (1949). The connective tissue sheath of nerve as effective diffusion barrier. *J. cell. comp. Physiol.* **34**, 1–16.

Hodgkin, A. L. (1949). Discussion to the communication on the membrane potential of frog nerve presented by R. Lorente do Nó to the International Conference on Neurophysiology, Paris, March-April in Proceedings. *Arch. Sci. physiol.* **3**, 237, no. 3, 367.

Huxley, A. F. & Stämpfli, R. (1951). Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibres. *J. Physiol.* **112**, 496–508.

Key, A. & Retzius, G. (1876). *Studien in der Anatomie des Nervensystems und des Bindgewebes*, Part 2. Stockholm: Samson and Wallis.

Lorente de Nó, R. (1947). A study of nerve physiology. *Stud. Rockefeller Inst. med. Res.* nos. 131 and 132.

Lorente de Nó, R. (1950). The ineffectiveness of the connective tissue sheath of nerve as a diffusion barrier. *J. cell. comp. Physiol.* **35**, 195–240.

Masson, P. (1942). Tumeurs encapsulées et benignes de Nerfs. *Rev. Canad. Biol.* **1**, 209–343.

Mullins, L. J. (1950). Uptake of phosphate by frog axon. *Fed. Proc.* **9**, 93.

Ranvier, L. A. (1878). *Leçons sur l'Histologie du Système Nerveux*, Vol. i. Paris: F. Savy.

Rashbass, C. & Rushton, W. H. H. (1949). The contribution of connective tissue impedance to the spatial spread of excitability in the frog's sciatic trunk. *J. Physiol.* **108**, 42 *P*.

Schoepfle, G. M. (1950). Electronic potentials elicited by threshold stimuli from sheath-free nerve at different temperatures. *Amer. J. Physiol.* **163**, 229–235.

Veall, N. (1948). A Geiger-Müller counter for measuring the beta-ray activity of liquids. *Brit. J. Radiol.* **21**, 347–351.

Young, J. Z. (1942). The functional repair of nervous tissue. *Physiol. Rev.* **22**, 318–374.

EXPLANATION OF PLATE

(All figures from specimens fixed in Flemming's fluid.)

Fig. 1. Transverse section of a multifascicular mammalian nerve showing the distribution of the connective tissue. Stained Weigert, × 100.

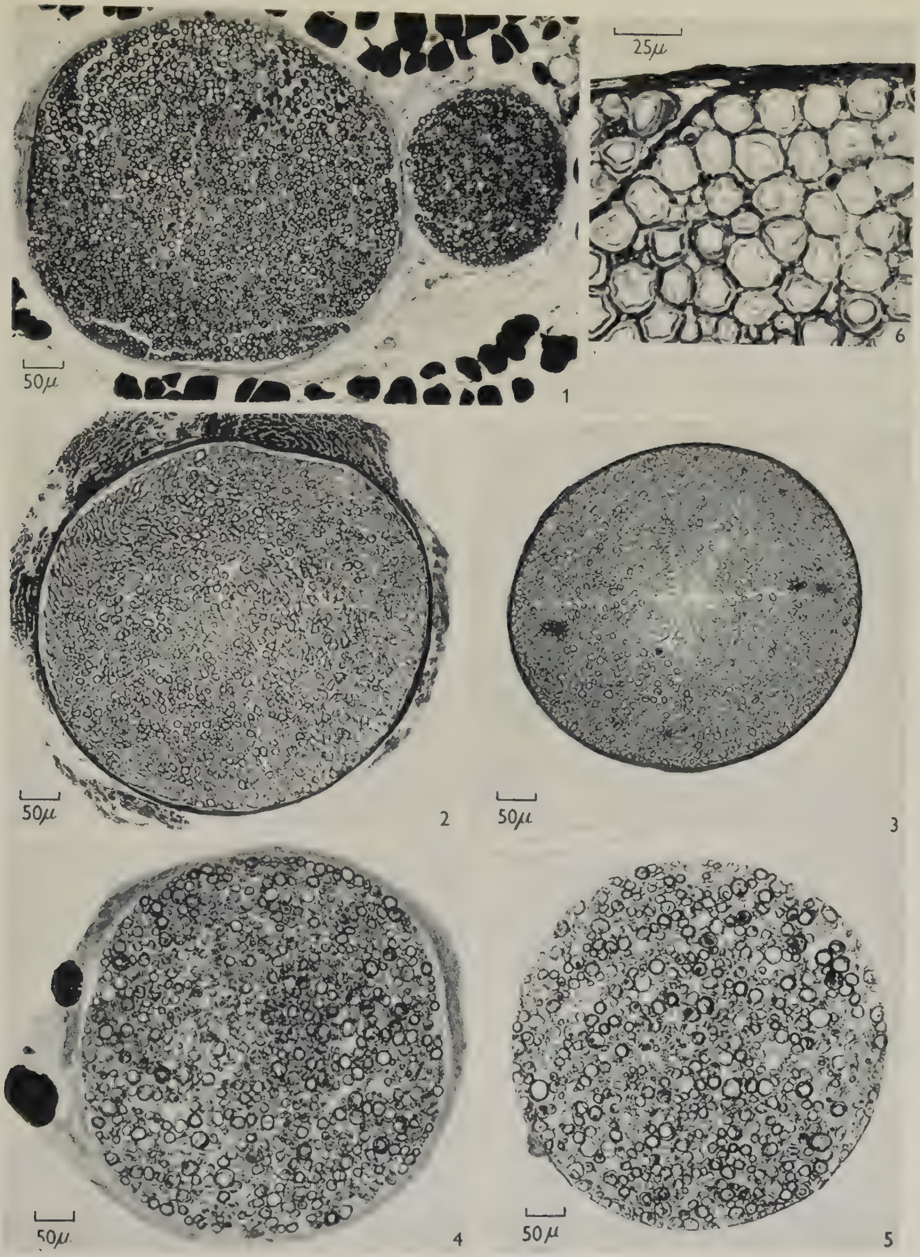
Fig. 2. Frog sciatic nerve with epineurium intact. Stained Weigert, × 100.

Fig. 3. A part of the same nerve as in Fig. 2 after the epineurium had been stripped. The perineurium remains in place. Stained Weigert, × 100.

Fig. 4. Rabbit peroneal nerve with epineurium intact. Stained Weigert, × 200.

Fig. 5. Rabbit peroneal nerve after stripping off the epineurium. The perineurium remains in place. Stained Weigert, × 100.

Fig. 6. Part of the periphery of a rabbit's sural nerve after removal of the epineurium. The inner lamellae of the perineurium are continuous with the endoneurium. Stained Azan, × 720.



CAUSEY AND PALMER—THE EPINEURAL SHEATH OF A NERVE AS A BARRIER TO THE DIFFUSION OF PHOSPHATE IONS

AN ELECTROMYOGRAPHIC STUDY OF THE TEMPORALIS AND MASSETER MUSCLES

By J. D. B. MacDOUGALL and B. L. ANDREW
The Departments of Anatomy and Physiology, University College, Dundee, University of St Andrews

INTRODUCTION

In his account of the movements of the mandibular joint, Robinson (1946) has drawn attention to differences of opinion concerning the actions of the muscles of mastication. Moyers (1950) made an extensive electromyographic study of this problem, and the introduction to his paper contains a review of the early literature on electromyography and an account of various techniques which have been used to study the mechanism of mastication. The electromyograph has also been used by Neumann (1950) to assess the overall activity of the masticatory muscles during the chewing of foods of various consistencies, but his electrodes were placed so widely apart that the action potentials of the components of the masseter and temporalis muscles were combined electrically.

In the present investigation, we had three main objectives, the primary one being to test the accuracy of Keith's statement (1920), that during an edge-to-edge bite on the incisor teeth the masseter is in a state of contraction and the temporalis is 'quite out of action', while on the other hand molar mastication is 'wholly a temporal act'. Palpation of the muscles during each kind of bite failed to convince us that their actions were as sharply divided from each other as Keith had suggested.

Our second aim was to examine the actions of the various components of the masseter and temporalis about which there has been disagreement in the past; for example, McCollum (1943) stated that the anterior fibres of the temporalis are active in protraction of the mandible, but Robinson (1946) denied this and further maintained that the masseter also plays no part in protraction.

Thirdly, we wished to investigate the possibility that the contraction of antagonistic muscles, rather than mere passive insufficiency or ligamentous action, plays a part in the limitation of movement at the mandibular joint. An action of this kind in an antagonistic muscle has already been reported by Mills (1950), who found that at the limit of maximal voluntary inspiration the obliquus externus abdominis and rectus abdominis became active.

METHODS

The comparative degree of contraction of the muscles was gauged from the alternating electrical disturbances picked up from the skin of the face and neck, the subjects examined being twelve normal adults between the ages of 20 and 35 years. The electrodes used were the points of two silver wires 0·018 in. in diameter and separated by 0·1 in. The wires were held by an insulator so that they pressed on the skin surface at right angles, adhesive tape being used to anchor them when necessary.

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Surface electrodes were the obvious choice for recording from the temporalis muscle because of its relatively isolated and superficial situation, and an advantage of the electrode described was that it could be used on a hairy surface. Furthermore, Moyers (1950) found that needle electrodes markedly interfered with the action of this muscle. By parting the hair with a comb it was possible to bring the points of the electrodes into contact with the skin over the temporalis without touching the hair, so avoiding the necessity of shaving the scalp. In our experiments on occlusion the pattern of electrical activity over the muscles was plotted on the skin surface, and for this it was necessary for the electrode to have a localized pick-up. As a large number of separate positions was being tested it was not desirable to insert electrode needles into the muscles. The pick-up of the electrode used was sufficiently localized to record the activity of single motor units in some favourable positions (Fig. 1).

Movement artefacts were distinguishable from muscle-action potentials, and to investigate other possible errors obtained with the wire electrodes, the ten electrode positions shown in Fig. 2 were tested for each movement with a different type of electrode which consisted of two strips of silver foil (1 × 0·25 cm.) separated by 0·2 cm. The results were qualitatively similar to those obtained with the wire electrode, although the pick-up was greater and more dependent on the orientation of the electrodes relative to the direction of the underlying muscle fibres, but the strip electrode was not suitable for use over hairy skin unless it had been previously shaved, and was not convenient for scanning considerable areas. Paired surface electrodes over the temporalis and masseter muscles were used by Pruzansky (1952) in his electromyographic investigation of normal and abnormal chewing patterns. Electrodes similar to our wire type were used by Gordon & Holbourn (1948).

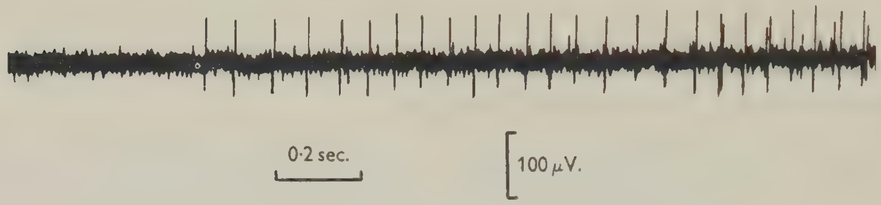

Fig. 1. Muscle-action potentials obtained from electrodes placed over the anterior fibres of the temporalis, showing the activity of individual motor units when a bite on the incisor teeth steadily increased from zero at the beginning of the record, which reads from left to right.

All our records were taken with the subject shielded in an earthed electrostatic screen. The electrodes were connected to the differential input stage of a capacity-coupled valve amplifier of conventional design. The output was displayed on a cathode-ray tube and also delivered audibly through a loud-speaker. Photographic recordings of the cathode-ray tube display were made as required for permanent records. The subject was earthed through an electrode in the mouth or through a band of saline soaked lint round the neck.

Electrodes over the procerus muscle served as control leads and showed only basic resting activity throughout the tests, except when the muscle was voluntarily contracted by wrinkling the skin over the root of the nose. Control leads were necessary in order to recognize any extraneous electrical disturbance which might have been circulating in the head as a whole, and the electrodes were placed over a facial muscle in order that they could be shown to be capable of picking up electrical disturbances by performing a voluntary contraction of that muscle. Although any facial muscle might have served the purpose, in practice, the procerus was found to be the most suitable. An associated action of the buccinator during occlusion for example, gave rise to typical waveforms which might have obscured the extraneous disturbances if they had been present.

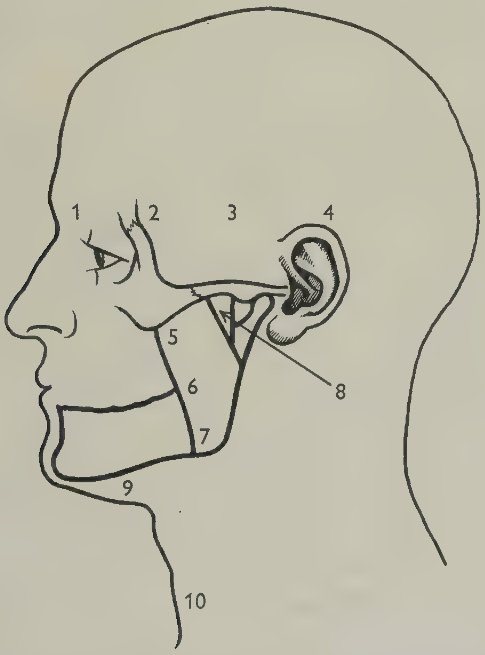

Fig. 2. Electrode positions. 1, control over the procerus muscle; 2, anterior fibres of the temporalis; 3, intermediate fibres of the temporalis; 4, posterior fibres of the temporalis; 5–7, superficial head of the masseter; 8, deep head of the masseter; 9, suprahyoid muscles; 10, infrahyoid muscles.

The procedure for plotting the activity of the masseter and temporalis muscles during occlusion was as follows. The subject held a thin piece of rubber tubing between his teeth and made the appropriate biting movement on request, while the experimenter applied the electrodes to spots at 0·5 cm. intervals over these muscles. The electrical activity at each spot was assessed either from the loudness of the sound from the loud-speaker or from the size of the waveforms on the cathode-ray tube. Only three grades of activity were designated, viz. (*a*) basic activity as found in the muscle at rest, (*b*) maximum activity, (*c*) any degree of activity which clearly lay between these extremes. Audible assessment by means of a loud-speaker was found to be easier than inspection of waveforms on the cathode-ray tube. A symbol indicating the electrical activity was then written on the tested spot. A total of 400–500 spots was tested, and the most active zones painted black. Typical photographs are shown in Fig. 3. The 'electrical image' indicated was found to overlie approximately the projection of the muscle on the skin surface obtained by direct palpation during alternate contractions and relaxations, but some spread, probably due to associated contraction of neighbouring muscles, was encountered.

For other parts of the work the electrical activity was recorded at selected positions, each overlying a portion of a muscle component which could be accurately located by palpation. These electrode positions are shown in Fig. 2.

The presence or absence of activity above the basic resting value at each of these positions during various movements was observed, and the findings for all the subjects are summarized in Table 1.

RESULTS

It was thought that the silver wire electrodes might have been too sensitive, in that small changes of position would alter the record owing to the presence of varying amounts of subcutaneous fat and tendinous tissue in the masseter; indeed this is the probable explanation of the fact that the superficial head of the masseter consistently showed more activity at its lower than at its upper part during some movements. The variable size of the parotid gland, and therefore of its shielding effect, may have been reflected in the occasional occurrence of a drop in electrical activity over the middle of the masseter as compared with its upper and lower parts. Nevertheless, this localized pick-up was essential when many separate but closely adjacent positions were being tested.

At rest

Weddell, Feinstein & Pattle (1944) observed that normal facial muscles constantly displayed tone demonstrable by slight electrical activity, and on using surface electrodes we found there was also slight activity at rest in masticatory muscles.

Biting

Two biting actions were studied, first, direct occlusion of the incisor teeth, and secondly, direct occlusion of the molars. Occlusion of these teeth was also studied with a hard object separating them by 0·5 cm.

Fig. 3*a* shows an example of the type of 'electrical image' found during incisor occlusion. It will be noted that while almost all of the area over the masseter muscle showed maximum activity, only a small part of the area over the anterior fibres of the temporalis did so. The image over the temporalis is incomplete, but when tested, even less activity was present over the middle and posterior fibres of this muscle than over its anterior fibres, although this cannot easily be presented graphically owing to the presence of hair.

The pattern of activity over these muscles in the same subject during occlusion of the molar teeth is shown in Fig. 3*b*. Although the masseter area showed some small changes, the muscle was certainly far from being out of action as suggested by Keith, but the amount of maximum activity over the temporalis was considerably increased, and if it could all be shown would extend backwards over the middle and posterior fibres of the muscle.

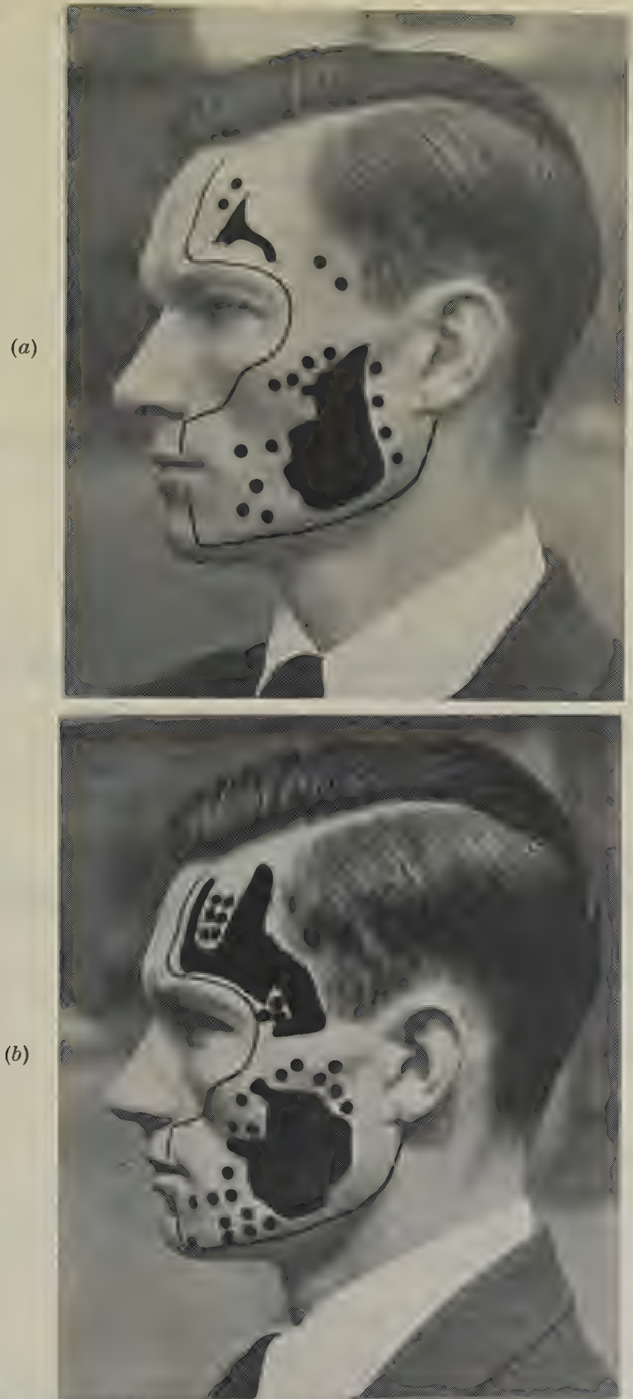

Fig. 3. 'Electrical images' obtained during each type of occlusion: (a) during incisor occlusion; (b) during molar occlusion. The solid black areas represent the most active zones, the spotted areas the less active zones, while the unmarked areas within the continuous line which delimits the area tested, indicate zones which showed basic resting activity only.

That this increase in the activity of the temporalis muscle when changing from incisor to molar occlusion was not merely due to a difference in the effort exerted by the subject is demonstrated in the electromyograms shown in Fig. 4, where a simple spring device producing a tooth separation of approximately 0·5 cm. was used to ensure that the bites were of equal force, in this instance 2 kg. The record shows that during molar occlusion something like twice as much activity was present as during incisor occlusion.

The presence of a small object between the teeth made little difference to the record obtained during biting. As might be expected, the activity in the masseter and temporalis during the movement of closing the jaw against gravity alone was slight when compared with that which began when the teeth met and the muscles contracted isometrically.

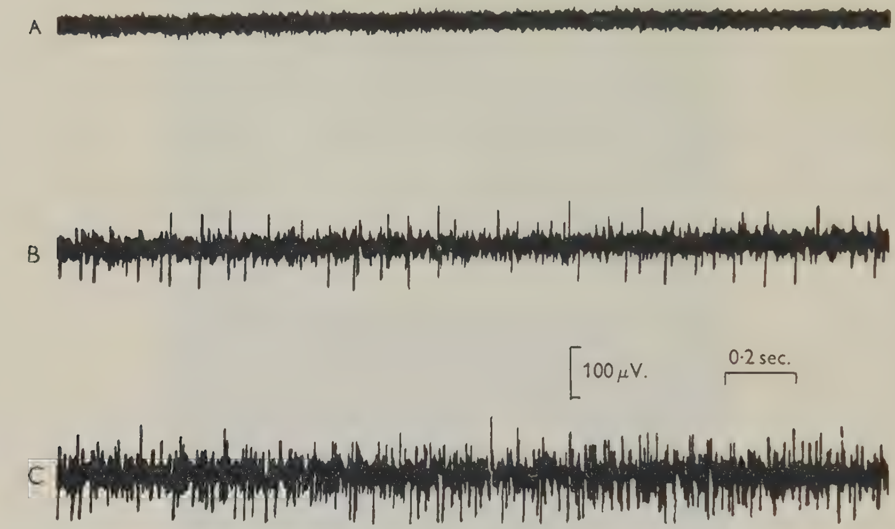

Fig. 4. Muscle-action potentials obtained from electrodes placed over the anterior fibres of the temporalis and strapped in position throughout. (A), at the position of rest; (B), during a bite on the incisor teeth with a force of 2 kg.; (C), during a bite on the molar teeth with a force of 2 kg. The records read from left to right.

Protraction

During the act of protracting the mandible, considerable activity was recorded over both heads of the masseter. Activity from individual motor units only was occasionally found over the anterior fibres of the temporalis. All other muscle components examined showed only basic resting activity.

Retraction

This action was carried out against the steady pressure of the observer's thumbs placed behind the angles of the subject's protracted mandible. Maximum activity was recorded over the posterior fibres of the temporalis and in some cases there was activity above the basic value over the deep head of the masseter.

Depression of the mandible

During unopposed mouth opening basic activity only was detected over the masseter and temporalis muscles. However, when maximal mouth opening was attempted, the electrical activity in these muscles rose to high levels at the terminal part of the movement. This effect was most marked over the anterior fibres of the temporalis and over the superficial head of the masseter. Approximately 1 sec. after the onset of such activity the subject experienced pain, poorly localized, in the region of the mandibular joint and ramus. Fig. 5 shows electrical records taken from the anterior fibres of the temporalis during maximal mouth opening in which the time of onset of pain is indicated. The point of onset of pain was necessarily only approximate, as discomfort preceded and merged into the sensation of pain.

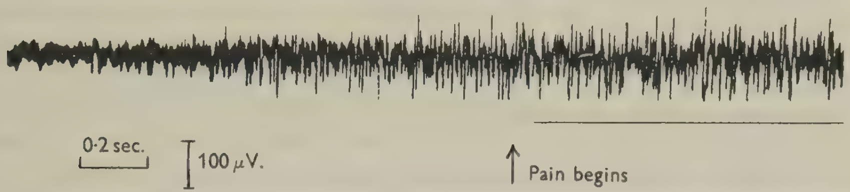

Fig. 5. Muscle-action potentials obtained at the end of maximal mouth opening from electrodes placed over the anterior fibres of the temporalis. The signal line below the record indicates pain felt by the subject. The record reads from left to right.

Table 1. *Summary of findings in all subjects, showing the activity over the various muscle components during different movements*

(The symbol + indicates activity above the basic resting value which is shown by the − sign. Where the findings were variable this is indicated by the ± sign.)

				Masseter						
	Temporalis			Superficial head						
	Anterior	Middle	Posterior	Upper	Middle	Lower	Deep head	Supra-hyoid	Infra-hyoid	Procerus
At rest	−	−	−	−	−	−	−	−	−	−
Protraction	−	−	−	+	+	+	+	−	−	−
Retraction against resistance	−	−	+	−	−	−	±	−	−	−
Opening the mouth	−	−	−	−	−	−	−	−	−	−
Opening the mouth maximally	+	+	±	+	+	+	+	+	+	−
Opening the mouth against resistance	−	−	−	−	−	−	+	+	+	−

The supra- and infrahyoid muscles became active when mouth opening was attempted against resistance. During the same movement, activity above the basic value was detected over the deep head of the masseter. Slight activity in the suprahyoid group occurred during the early part of unopposed mouth opening. This is not shown in Table 1.

DISCUSSION

The part of this study dealing with muscle activity patterns during biting with the incisor and the molar teeth was designed to test the statement that the masseter is entirely responsible for biting with the incisors, while the temporalis alone is active in molar occlusion. Our results can give only partial support to this view, as we found that, while in occlusion of the incisor teeth the masseter is more active than the temporalis, in molar occlusion both muscles are involved approximately to the same extent. This may be associated with the fact that when the teeth are in the incisor relationship, the mandible is slightly protracted and the direction of the fibres of the temporalis muscle is less direct. Our findings, therefore, are not in agreement with the suggestion of Keith (1920) that modern man with his overlapping bite and diet of soft food practically uses only his temporal muscle, the masseter being 'hardly used at all'.

The activity observed over the masseter during protraction might be interpreted as due to the fact that this muscle directly aids in protraction, or as suggested by Moyers (1950) that it acts to prevent the lateral pterygoid from opening the mouth during protraction. Robinson (1946) maintained that the masseter is unable, for mechanical reasons, to protract the mandible. If the masseter is only a synergist during protraction, however, a similar action might be expected in the temporalis, and this was not observed, the occasional slight activity over the anterior fibres of this muscle being regarded as insignificant. The evidence is not conclusive therefore, but it is probable that the masseter aids in protraction directly as well as having a synergic action, while the anterior fibres of the temporalis play little part, if any, in the same movement.

Our findings during retraction of the mandible confirmed the view that the posterior fibres of the temporalis are the main agents in this movement. Participation of the deep head of the masseter in retraction, which might be expected from the direction of its fibres, downwards and forwards during full protraction, was not constantly demonstrated.

The results obtained during unopposed submaximal depression of the mandible suggested that this movement is largely due to the action of gravity. The suprahyoid muscles, however, appeared to play some part in the initiation of mouth opening.

During depression of the mandible against resistance, the activity observed over the deep head of the masseter was unexpected. It is unlikely that this was due to an increase in tone caused by stretching of the muscle, as the superficial head of the masseter showed only basic resting activity, and in addition the resistance was maintained in such a way that only slight tooth separation was permitted. The electrodes placed over the deep head of the masseter were also directly over the mandibular notch and therefore no bone intervened between the electrodes and at least the upper head of the lateral pterygoid. Because of these facts, we would suggest that during attempted mouth opening against resistance, and probably also during protraction, the activity observed over the deep head of the masseter was derived from the underlying lateral pterygoid.

The activity over the temporalis and masseter at the end of maximal mouth opening is of interest. This activity preceded the onset of pain by a small time margin, and one possible explanation of the effect is that the action of these antagonistic muscles is protective and sets a limit to voluntary action. In the ordinary course of events, contraction of the temporalis and masseter probably prevents further mouth opening at the stage where pain is not yet present; indeed it requires an effort of will to force the mouth open to the stage where pain is experienced. Although Moyers (1950) does not refer to this in the text of his paper the effect is indicated in his tracings taken during mouth opening. Partridge (1924) suggested on morphological evidence that antagonist muscles operated to prevent joint injury. It may be significant that dislocation of the mandibular joint usually follows an excessive *involuntary* action such as laughing or yawning.

SUMMARY

1. The actions of the temporalis and masseter muscles were studied in twelve subjects by means of the electromyograph, using surface electrodes.

2. In occlusion of the incisor teeth, activity was mainly centred over the masseter muscle, and to a lesser extent over the temporalis. In occlusion of the molar teeth, activity was more or less equal over all parts of the masseter and temporalis muscles.

3. Protraction was accompanied by considerable activity over the masseter. Retraction against resistance led to activity over the posterior fibres of the temporalis and less constantly over the deep head of the masseter.

4. During ordinary unforced mouth opening no activity beyond the basic resting value was recorded over the masseter and temporalis nor, in the greater part of the movement, over the supra- and infrahyoid muscles. Maximal mouth opening, however, resulted in considerable activity over all these muscle groups. In the case of the masseter and temporalis muscles, this may have been a protective action to prevent dislocation of the mandibular joint. Attempted opening of the mouth against resistance was accompanied by activity over the supra- and infrahyoid muscles.

We wish to thank Prof. D. R. Dow and Prof. G. H. Bell for their help and advice. The patient co-operation of students and members of staff who acted as subjects for our investigations is also gratefully acknowledged.

REFERENCES

Gordon, G. & Holbourn, A. H. S. (1948). The sounds from single motor units in a contracting muscle. *J. Physiol.* **107**, 456–464.

Keith, A. (1920). In a discussion on a paper by L. H. Dudley Buxton 'The teeth and jaws of savage man'. *Trans. Brit. Soc. Orthodont.* 1916–20, pp. 79–88.

McCollum, B. B. (1943). Oral diagnosis. *J. Amer. dent. Ass.* **30**, 1218–1233.

Mills, J. N. (1950). The nature of the limitation of maximal inspiratory and expiratory efforts. *J. Physiol.* **111**, 376–381.

Moyers, R. E. (1950). An electromyographic analysis of certain muscles involved in temporomandibular movement. *Amer. J. Orthodont.* **36**, 481–515.

Neumann, H. H. (1950). Electrical action currents during mastication. *J. dent. Res.* **29**, 463–468.

Partridge, E. J. (1924). Joints. *J. Anat., Lond.*, **58**, 346–354.

Pruzansky, S. (1952). The application of electromyography to dental research. *J. Amer. dent. Ass.* **44**, 49–68.

Robinson, M. (1946). The temporomandibular joint; theory of reflex controlled non lever action of the mandible. *J. Amer. dent. Ass.* **33**, 1260–1271.

Weddell, G., Feinstein, B. & Pattle, R. E. (1944). The electrical activity of voluntary muscle in man under normal and pathological conditions. *Brain*, **67**, 178–257.

OBSERVATIONS UPON CERTAIN REPUTED ACCESSORY NUCLEI OF THE OCULOMOTOR COMPLEX

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INTRODUCTION

Following the fundamental work of Stilling (1846) and von Gudden (1881), who established the main or somatic oculomotor nucleus as the principal source of the axons of the third nerve, evidence has been advanced from time to time to include certain other nuclei, in the vicinity of the oculomotor, as accessory to it. The Edinger-Westphal and antero-median nuclei, usually regarded as the parasympathetic components of the oculomotor complex, were early defined; but they have attracted a volume of controversy and will not be considered in this brief account.

The nucleus which Darkschewitsch (1889) first identified in human foetal material was regarded as an element of the oculomotor group by Perlia (1889), Tsuchida (1906), Mingazzini (1928), and others. Knies (1891) went so far as to state that it innervated the sphincter pupillae. Evidence, mostly pathological, that it does not contribute oculomotor fibres, has been reported by von Kölliker (1896), von Bechterew (1897), Bernheimer (1897), Panegrossi (1898), Bach (1899), Juliusberger & Kaplan (1899), von Reusz (1899), and Ariens Kappers (1920). Zeri (1895), Panegrossi (1898), and Ramón y Cajal (1909) subdivided the original nucleus of Darkschewitsch into a small-celled dorso-medial part, which retains the eponym, and a ventrolateral mass, composed of larger cells, named the nucleus interstitialis by Ramón y Cajal (1909). Stengel (1924) and Ingram & Ranson (1935) reviewed the literature concerning these nuclei; they did not support their inclusion in the oculomotor complex. Nevertheless, both are still frequently described and figured in association with the established sources of oculomotor fibres.

The nucleus dorso-centralis of Panegrossi (1898), said to be invariably involved in the pathological changes of chronic ophthalmoplegia both by him and by Mingazzini, corresponds in all particulars with the caudal central nucleus of Tsuchida (1906). The last worker, Le Gros Clark (1926), and subsequent authorities, have described this nucleus as part of the oculomotor group in primates.

In a pathological report on a case of ophthalmoplegia, Frank (1921) recorded changes in the cells of the dorsal nucleus of the raphé, and concluded that it was concerned in the innervation of the intrinsic ocular musculature. He noted similar changes in a crescentic group of cells, ventral to the medial longitudinal fasciculus at trochlear levels, which he called the subfascicular nucleus. This mass appears to be identical with the annular nucleus of Ziehen (1920) and Castaldi (1923), as Ariens Kappers, Huber & Crosby (1936) have pointed out. Mingazzini (1928) agreed with Frank concerning both these nuclei, but no further observations upon their relation to the oculomotor centres have appeared.

Ferrier (1863), Duval & Laborde (1880), and Woods (1884) described clinical and physiological evidence suggesting the existence of a contribution of ascending abducent axons to the oculomotor nerve to innervate the rectus medialis. Clarke (1868) early denied this, and most subsequent workers have found no evidence of such fibres. More recently, Scala & Spiegel (1933) have re-opened this old controversy by reporting physiological observations which, they believed, confirmed the presence of fibres ascending in the medial longitudinal fasciculus of the cat to supply the rectus medialis.

Since most of the nuclei mentioned above have been associated by various authorities with the recognized sources of oculomotor axons, the opportunity was taken of examining the condition of their cells in a large series of monkeys subjected to trunk and branch interruptions of the oculomotor nerve.

MATERIAL AND METHODS

Serial sections of the midbrain and pons of monkeys (*Macaca mulatta* and *Cercocebus fuliginosus*) were inspected for retrograde changes in the cells of the putative oculomotor nuclei enumerated above, after unilateral intracranial division of the third nerve (six animals) and extirpation of individual extra-ocular muscles (fifty-five animals). At periods varying from 8 to 18 days after operation the animals were killed and perfused. Paraffin sections, 10 μ in thickness, were stained either with gallocyanin or cresyl fast violet. The condition of each nucleus was observed in at least fifty separate midbrains, except in the case of the abducent, which was concerned in twenty-one experimental series. Serial sections of the oculomotor complex in five normal monkeys were also examined.

RESULTS

The nucleus of Darkschewitsch

This nucleus appeared in sections through the cranial fifth of the oculomotor complex, lying dorso-lateral to the somatic column and lateral to the Edinger-Westphal nucleus (Pl. 1, fig. 1). It extended somewhat cranially beyond the somatic column. Its cells were small and variable in form, some being multipolar and like those of the interstitial nucleus, but in general their form was more rounded. Their chromatin granules varied in size and intensity of staining, and since their total chromatin content was commonly less than that of the somatic oculomotor neurones, they were usually comparatively pale cells (Pl. 2, fig. 5). The granules in them were frequently arranged at the periphery of the cell, and in some series this appearance was so marked as to resemble chromatolysis; but no other evidence of retrograde degeneration was seen in any of the sixty-one series examined. Furthermore, no differences between the cells in the right and left nuclei of Darkschewitsh were observed, even in preparations from animals subjected to complete and unilateral oculomotor division. In every series the condition of the cells was uniform, not merely in both nuclei but also among the cells of each. Whenever pallor was marked it affected most of the cells, and showed no relationship to the side or magnitude of the procedures carried out.

Nucleus interstitialis

In the monkey this nucleus was seen to extend through about the cranial third of the oculomotor complex, lying lateral to the dorsal part of the somatic nucleus (Pl. 1, fig. 1). Its nerve cells were larger than those of the nucleus of Darkschewitsch but smaller than somatic oculomotor cells. They were markedly multipolar, with long processes, and contained variable amounts of chromatin material. In most series the cells contained large, discrete Nissl granules (Pl. 2, fig. 6), but in some the majority displayed a faintly stained reticulum in the cytoplasm. The dust-like disintegration of granules typical of retrograde degeneration in somatic motor nerve cells was not observed, although the pale appearance of the cells did occasionally resemble chromatolysis. As in the case of the nucleus of Darkschewitsch the distribution and number of such pale cells was quite unrelated to the extent of the nerve interruptions which had been carried out. In the first place, the state of the cells of right and left interstitial nuclei was always identical, despite the unilateral nature of all the experimental lesions; and secondly, pale cells, if present, were predominant, and proportionately equal on both sides, irrespective of the magnitude of the axonal lesion.

The caudal central nucleus

Evidence has been reported elsewhere (Warwick, 1951) to show that the caudal central nucleus contributes fibres to the oculomotor nerves. This median mass of motor cells, situated dorsal to the somatic oculomotor nuclei in somewhat more than their caudal third, is well developed in the monkey (Pl. 1, fig. 2). Its cells are intermediate in size between those of the somatic and Edinger-Westphal nuclei, multipolar in shape, and contain large densely staining granules (Pl. 2, fig. 7). Following oculomotor division (six animals) and extirpation of the levator (fifteen animals), many of the cells of this nucleus showed retrograde changes (Pl. 2, fig. 8). In forty series, prepared from animals subjected to resection of other muscles supplied by the third nerve, no such effects were observed.

The dorsal nucleus of the raphé

This median group, easily identified in monkey midbrains, was present in sections at the oculomotor-trochlear junction and in all which passed through the trochlear nuclei. It was quite separate from the caudal central nucleus, lying between this and the aqueduct (Pl. 1, fig. 2). Its constituent cells were smaller and usually more rounded than those of the caudal central nucleus, and their chromatin granules were sparsely distributed and often peripherally condensed (Pl. 2, fig. 9). The consequent pale appearance of these cells, together with frequent nuclear eccentricity, produced in some series a resemblance to the earlier phases of retrograde degeneration, which was, however, shared by almost all the cells of the nucleus, irrespective of the scale of axonal injury, and despite the fact that all the experimental interruptions of the oculomotor nerve were unilateral. Examination of the cells of this nucleus in five normal series showed similar characteristics; it was not possible to distinguish between the cells of the nucleus in normal or experimental series.

The subfascicular nucleus

Small groups of nerve cells, closely applied to the ventral aspect of the medial longitudinal fasciculi at trochlear levels, were present in all the monkey midbrains examined (Pl. 1, fig. 3). They appeared to be continuous with the caudal linear nucleus, from which they spread out crescentically, right and left, diminishing rapidly in a lateral direction. These groups resembled the descriptions of the annular nucleus cited above. The identity of Frank's subfascicular nucleus with the ventral part of this was accepted here, since no other nuclear mass answering his description could be found in this region. The cells of the subfascicular nuclei were similar to those of the dorsal nucleus of the raphé (Pl. 2, fig. 9), being rounded, small and containing a few large chromatin granules, which were often disposed chiefly near the surface of the cell. This kind of cell was also found scattered in small numbers on all aspects of the trochlear nuclei and medial longitudinal fasciculi; the dorsal nucleus of the raphé, the caudal linear and subfascicular nuclei appeared indeed to be localized clusters of such cells. The appearance of these cells was the same, both in experimental and normal material, and no selective changes, such as might be expected from the unilateral and partial oculomotor lesions which had been carried out, were noted in any series. No indisputable evidence of retrograde degeneration was ever observed in the cells of the subfascicular nucleus, even after total oculomotor division.

The abducent nucleus

Examination of both abducent nuclei in serial sections from six monkeys submitted to unilateral division of the oculomotor nerve yielded no evidence of retrograde degeneration in their cells. Similarly, the results after ten extirpations of the rectus medialis were negative, nor were retrograde effects ever seen in the abducent nucleus after resection of any other extra-ocular muscle except the rectus lateralis. Resection of the latter muscle in five animals produced marked chromatolysis in almost all the motor cells of the ipsilateral nucleus (Pl. 2, fig. 10), and hence the negative effects of oculomotor lesions could not have been due to any difficulty in obtaining a retrograde response.

DISCUSSION

Of the five supposed accessory oculomotor nuclei considered above, one only was confirmed as a source of third nerve fibres, this being the caudal central nucleus (nucleus posterior dorso-centralis of Panegrossi). This experimental result is in agreement with the clinico-pathological views of Panegrossi (1898), Tsuchida (1906) and Mingazzini (1913), and with the comparative observations of Le Gros Clark (1926). The last authority suggested an association between this nucleus and the Edinger-Westphal columns, reputedly of autonomic function; but it was found in the material of this report that the caudal central nucleus innervates a somatic muscle, the levator palpebrae superioris. It is true that this muscle has associated with it a stratum of unstriped fibres, the superior palpebral muscle, but this is said to be innervated by the cervical sympathetic via the cavernous plexus (Whitnall, 1932).

The negative findings reported here in connexion with the nucleus of Darkschewitsch and nucleus interstitialis corroborate the views of most recent workers. Evidence associating both with the vestibular system has been advanced by Muskens (1914, 1937), Jakob (1923) and Lotmar (1926), and their close topographical relation to the oculomotor complex may indicate a participation in oculo-vestibular integration. Whatever their connexions with the oculomotor complex may be, the evidence of this research was that they are not radicular oculomotor nuclei. It is worthy of note that the poor staining of the cells of the nucleus of Darkschewitsch was reported by Ramón y Cajal (1909); Crosby & Woodburne (1943) regarded their chromatin granules as large and dense, but Ramón y Cajal's view was confirmed here.

With regard to the subfascicular nucleus and the dorsal nucleus of the raphé, the present findings, based upon considerable experimental material, do not substantiate the claims of Frank (1921) and Mingazzini (1928). The former's conclusions were founded solely upon a single case of ophthalmoplegia partialis, and Frank himself admitted the difficulty of interpreting the pathological changes in such chronic nuclear disease. Mingazzini did not specify the evidence upon which he found himself in agreement with Frank, who had worked in his laboratory. It appears, therefore, that their inclusion of these two nuclei in the oculomotor complex was insecure. The observations described here, based upon sixty-one interruptions of the branches and trunk of the oculomotor nerve, yielded no evidence that any of its fibres are derived from either the subfascicular nucleus or the dorsal nucleus of the raphé.

Neural mechanisms integrating the activities of the extra-ocular muscles and their nuclei have attracted much interest. The reciprocal innervation of the recti medialis et lateralis, classically demonstrated by Sherrington (1894), pointed clearly to the existence of connexions between the third and sixth nerve nuclei; but the conceptions of the earlier workers cited above differed concerning the integration of the medial and lateral recti of opposite orbits in binocular movements of conjugate deviation. The dual innervation of the rectus medialis, by oculomotor and abducent axons, which they hypothecated, received only apparent corroboration from the experiments of Scala & Spiegel (1933); the medial rotation of the opposite eye, which these latter workers claimed to have produced by unilateral stimulation of the medial longitudinal fasciculus at positive levels, was not proof that abducent fibres were involved or that the fibres stimulated did in fact proceed directly to the opposite rectus medialis. Moreover, Spiegel & Scala (1936) considered that these ascending fibres were derived from the vestibular nuclei. Whatever the nature of the connexions integrating the third and sixth nerve nuclei in lateral ocular movements, the results of this research indicated that no uninterrupted abducent axons pass out in the monkey's oculomotor nerve either to the rectus medialis or any other extra-ocular muscle.

It is concluded that of the supposed accessory sources of oculomotor axons discussed above only the caudal central nucleus can be regarded as a radicular component of the oculomotor complex. In particular, attention is drawn to the uncertain status of the nucleus of Darkschewitsch and the nucleus interstitialis in oculomotor function. There appears no clear reason, apart from topographical proximity, to support their frequently unexplained inclusion upon the outskirts of diagrams of the oculomotor centres.

SUMMARY

Various authorities have suggested the addition to the oculomotor complex of the nucleus of Darkschewitsch, the nucleus interstitialis (of Cajal), the dorsal nucleus of the raphé, and the subfascicular, caudal central and abducent nuclei, as accessory sources of third nerve axons. Examination of the cells of all these nuclei for retrograde changes, in serial sections of the brainstems of sixty-one monkeys which had been submitted to complete and partial interruptions of the oculomotor nerve, yielded positive findings only in the case of the caudal central nucleus. The paucity of evidence supporting any of the others as radicles of the oculomotor nerve has been discussed. Tacit inclusion of the nucleus of Darkschewitsch and nucleus interstitialis in diagrams of the oculomotor complex was regarded as especially misleading.

REFERENCES

Ariens Kappers, C. U. (1920). *Vergleichende Anatomie des Nervensystems.* Haarlem: E. F. Bohn.

Ariens Kappers, C. U., Huber, E. C. & Crosby, C. C. (1936). *The Comparative Anatomy of the Nervous System of Vertebrates, including Man.* New York: The Macmillan Company.

Bach, L. (1899). Zur Lehre von den Augenmuskellähmungen und den Störungen der Pupillenbewegung. *v. Graefes Arch. Ophthal.* **47**, 339–386 and 551–630.

Bechterew, W. von (1897). Ueber die Kerne der mit den Augenbewegungen in Beziehung stehenden Nerven (des Oculomotorius, Abducens und Trochlearis) und über die Verbindung derselben unter einander. *Arch. Anat. Physiol., Lpz.*, 307–315.

Bernheimer, S. (1897). Experimentelle Studien zur Kenntniss der Innervation der inneren und aüsseren Muskeln des Auges. *v. Graefes Arch. Ophthal.* **44**, 481–525.

Castaldi, L. (1923). Studi sulla struttura e sullo svilluppo del mesencefalo. *Arch. ital. Anat. Embriol.* **20**, 25–226.

Clarke, J. L. (1868). Researches on the intimate structure of the brain, second series. *Philos. Trans.* B, **158**, 263–331.

Crosby, E. C. & Woodburne, R. T. (1943). The nuclear pattern of the non-tectal portions of the midbrain and isthmus in primates. *J. comp. Neurol.* **78**, 441–482.

Darkschewitsch, L. (1889). Ueber die oberen kern des N. oculomotorius. *Arch. Anat. Physiol., Lpz.*, 107–116.

Duval, M. & Laborde, J. V. (1880). De l'innervation des mouvements associés des globes oculaires. *J. Anat., Paris*, **16**, 56–89.

Ferrier, D. (1863). Tumour of medulla oblongata. Remarks. *Brit. med. J.* ii, 821.

Frank, C. (1921). Über die Lokalisation in den Augenmuskel nervenkernen und zwei unbekannte Kerne im Mittelhirn des Menschen. *J. Psychol. Neurol., Lpz.*, **26**, 200–227.

Gudden, B. A. von (1881). Ueber die Kerne der Augenbewegungs nerven. *Zbl. Augenheilk.* **5**, 528–529.

Ingram, W. R. & Ranson, S. W. (1935). The nucleus of Darkschewitsch and nucleus interstitialis in the brain of man. *J. nerv. ment. Dis.* **81**, 125–137.

Jakob, A. (1923). *Die extrapyramidalen Erkrankungen.* Berlin: Julius Springer.

Juliusberger, O. & Kaplan, L. (1899). Anatomischer Befund bei einseitiger Oculomotoriuslähmung im Verlaufe von progressiver Paralyse. *Neurol. Zbl.* **18**, 486–495.

Knies, M. (1891). Über die zentralen Störungen der willkürlichen Augenmuskeln. *Arch. Augenheilk.* **23**, 19–51.

Kölliker, A. von (1896). *Handbuch der Gewebelehre des Menschen.* 6 Aufl. Leipzig: W. Engelmann.

Le Gros Clark, W. E. (1926). The mammalian oculomotor nucleus. *J. Anat., Lond.*, **60**, 426–448.

Lotmar, F. (1926). *Die Stammganglien und die extrapyramidal-motorischen Syndrome.* Berlin: Julius Springer.

Mingazzini, G. (1913). *Anatomia Clinica dei Centri Nervosi*, 2d. ed. Torino: Unione Tipografico Editrice Torinese.

Mingazzini, G. (1928). Medulla oblongata und Brücke. In G. von Mollendorf's *Handbuch der mikroskopische Anatomie des Menschen*, **4**, 650–651. Berlin: Julius Springer.

Muskens, L. J. J. (1914). An anatomical-physiological study of the posterior longitudinal bundle in its relation to forced movements. *Brain*, **36**, 352–426.

Muskens, L. J. J. (1937). Cortical innervation of ocular movements in the horizontal plane. *Arch. Ophthal., N.Y.*, **18**, 527–531.

Panegrossi, G. (1898). Contributo allo studio anatomo-fisiologico dei centri dei nerve oculomotori dell' uomo. *Ric. Lab. Anat. norm. Univ. Roma*, **6**, 103–155.

Perlia, R. (1889). Die Anatomie des Oculomotorius-centrums beim Menschen. *v. Graefes Arch. Ophthal.* **35**, 287–398.

Ramón y Cajal, S. (1909). *Histologie du Système Nerveux de l'Homme et des Vertèbres*, **2**, 262–264. French ed. by L. Azoulay. Paris; A. Maloine.

Reusz, F. von (1899). Beitrag zur pathologischen Anatomie der Bulbärkrankungen bei Tabes. *Arch. Psychiat.* **32**, 535–549.

Scala, N. P. & Spiegel, M. D. (1933). Effect of stimulation of the posterior longitudinal fasciculus on ocular muscles. *Arch. Ophthal., N.Y.*, **9**, 939–946.

Sherrington, C. S. (1894). Experimental note on two movements of the eye. *J. Physiol.* **17**, 27–29.

Spiegel, M. D. & Scala, N. P. (1936). The cortical innervation of ocular movements. *Arch. Ophthal., N.Y.*, **16**, 967–981.

Stengel, E. (1924). Vergleichend-anatomische Studien über die Kerne an der hinteren Kommissur und im Ursprungsgebiete des hinteren Längsbundels. *Arb. neurol. Inst.* (*Inst. Anat. Physiol. ZentNerv.*) *Univ. Wien*, **26**, 419–454.

Stilling, B. (1846). Disquisitiones de structura et functionibus cerebri. Jenae: sumt. F. Maukii. T.1, 36.

Tsuchida, U. (1906). Über die Ursprungskerne der Augenbewegungs-nerven im Mittel- und Zwischenhirn. *Arb. hirnanat. Inst., Zurich*, nos. 1–2, 1–205.

Warwick, R. (1951). A study of retrograde degeneration in the oculomotor nucleus of the rhesus monkey. *Brain*, **73**, 532–543.

Whitnall, S. E. (1932). *The Anatomy of the Human Orbit*, 2nd ed. p. 146. London: Oxford University Press.

Woods, G. A. (1884). The anatomy, etc., of the sixth nerve. *Lpool med.-chir. J.* **4**, 1–29.

Zeri, A. (1895). Sulla alterazione dei centri nervosi nella tabe. *Riv. sper. freniat.* **21**, 580–641.

Ziehen, T. (1920). Mikroskopische Anatomie des Gehirns. In K. von Bardeleben's *Handbuch der Anatomie des Menschen*, **4**, Abt. ii, T. 2. Jena: Gustav Fischer.

EXPLANATION OF PLATES

All figures are of fields in 10 μ transverse sections of the oculomotor complex of monkeys. All preparations were stained by Bielchowsky's dilute cresyl fast violet technique.

Plate 1

Fig. 1. The oculomotor complex near its rostral extremity, showing the nucleus of Darkschewitsch (*D*) and nucleus interstitialis (*I*). The Edinger-Westphal nucleus is also indicated (*EW*). × 30.

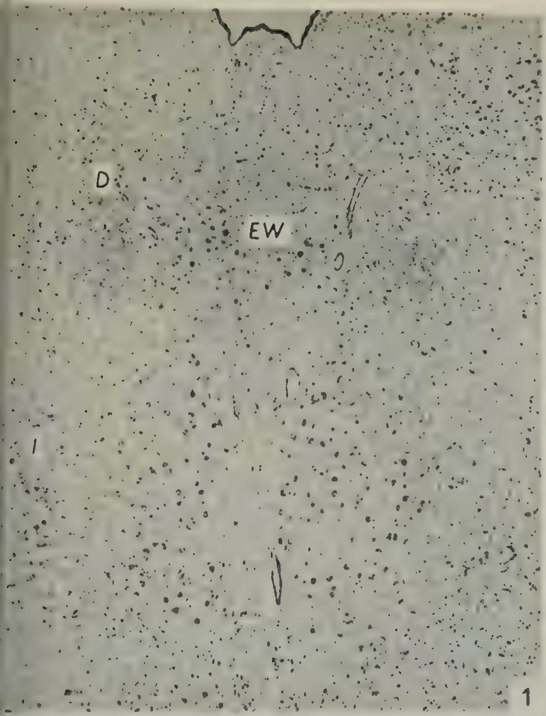
Fig. 2. A typical field in the caudal third of the oculomotor complex, showing the caudal central nucleus (*CCN*) and the dorsal nucleus of the raphé (*DNR*). × 30.

Fig. 3. Section through the trochlear nuclei, showing Frank's subfascicular nuclei (SF), the caudal linear nucleus (*CL*), and the dorsal nucleus of the raphé (*DNR*). The medial longitudinal fasciculus (*MLF*) is also indicated. × 35.

Fig. 4. Normal somatic oculomotor nerve cells. These do not show the large processes characteristic of the cells of the nucleus interstitialis (Pl. 2, fig. 6), but are of similar size and display a similar distribution of chromatin granules. × 550.

Plate 2

Fig. 5. Cells of the nucleus of Darkschewitsch. Contrast their small sparse chromatin granules, peripheral in distribution, with those in the somewhat larger cells of the nucleus interstitialis (Fig. 6). × 550.



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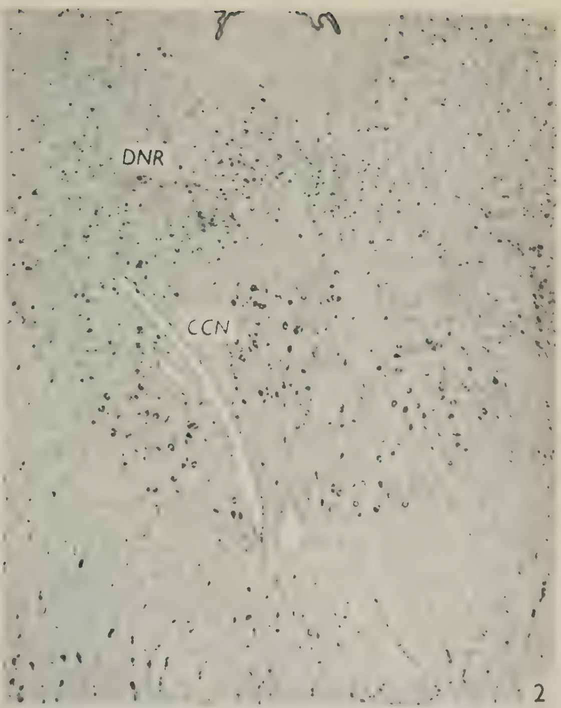

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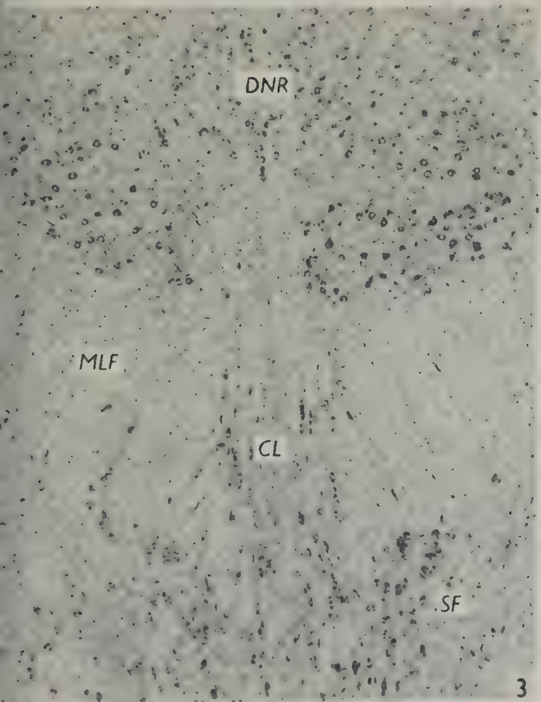

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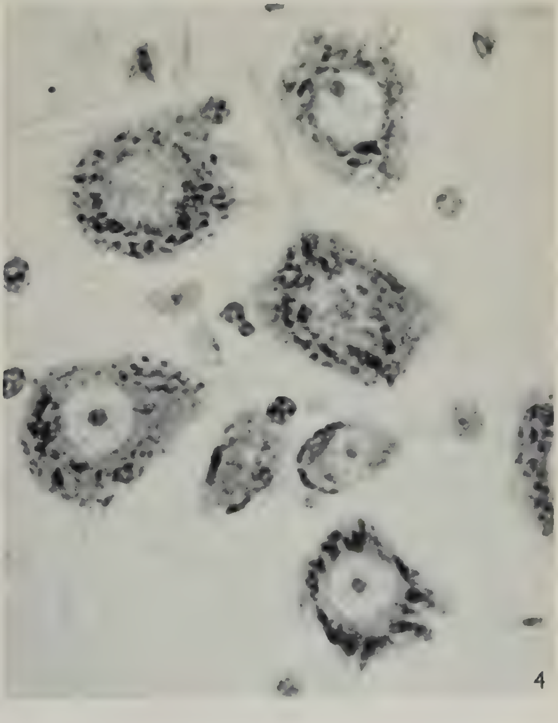
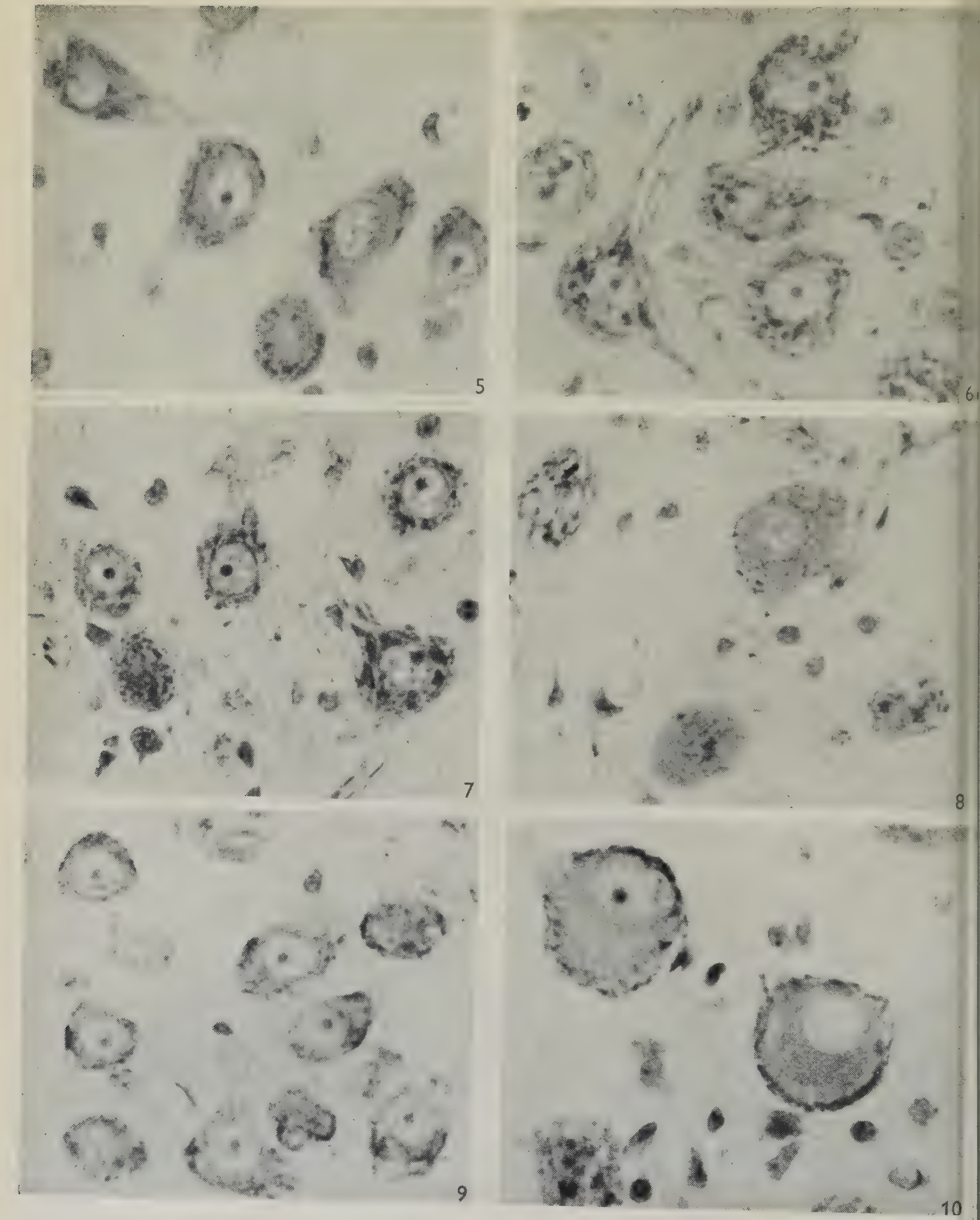


4

ARWICK—Certain reputed accessory nuclei of the oculomotor complex



WARWICK —Certain reputed accessory nuclei of the oculomotor complex

Fig. 6. Nerve cells of the nucleus interstitialis. The chromatin granules of these cells are not always so prominent as in this preparation, but the long processes are typical. × 550.

Fig. 7. Normal cells of the caudal central nucleus. Compare these with somatic oculomotor nerve cells (Pl. 1, fig. 4), which they closely resemble except in their slightly smaller size. Contrast them with the cells of the dorsal nucleus of the raphé (fig. 9). × 550.

Fig. 8. Retrograde degeneration in the cells of the caudal central nucleus eleven days after resection of one levator palpebrae superioris. Contrast the two central chromatolytic cells with the normal ones in fig. 7. × 550.

Fig. 9. Normal cells of the dorsal nucleus of the raphé, showing the peripheral disposition and condensation of their scanty content of chromatin material. This, the clear peri-nuclear zone, and also frequent nuclear eccentricity, produce a superficial resemblance to chromatolysis, but cells in normal material show the same characteristics. × 550.

Fig. 10. Nerve cells from the abducent nucleus, showing retrograde changes following resection of the ipsilateral rectus lateralis. The swelling, rotundity, eccentric nuclei, peri-nuclear chromatolysis, and clumping of granules at the periphery of the cells are typical of early retrograde degeneration in somatic motor neurones. × 550.

MORPHOGENETIC STUDIES OF THE RABBIT

XI. GENETIC DIFFERENCES IN THE GROWTH OF THE VERTEBRAL COLUMN AND THEIR RELATION TO GROWTH AND DEVELOPMENT IN MAN

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INTRODUCTION

Though a great deal has been written about the physical growth of the human, and about differences in body build between one person and another, comparable literature on other animals is extraordinarily sparse. This is somewhat surprising, since bodily differences, being largely genetic, are much more readily studied in laboratory stock. It is also unfortunate, in that lack of animal data prevents our knowledge of human growth and physique being placed in its proper biological perspective. This situation will only be remedied when complete longitudinal studies of the growth of animal stocks are undertaken and carried through, but meanwhile some animal material is available, and previous papers of this series have described, in inbred races of rabbits, genetically controlled differences in various morphological traits, chiefly as seen in the newborn. The present paper analyses the differences between the vertebral bodies of adult animals in three inbred races of rabbits. It reaches the conclusion, by way of analyses of variance, that these are best described in terms of factors for general size, for linearity, and for growth of particular regions, a result strikingly similar to that emerging from the factor-analysis classification of human physique.

MATERIAL AND METHODS

The material consisted of the vertebral skeletons of three inbred races of rabbits, races III, V and X. Race III is a New Zealand White rabbit, of average mature body weight about 3800 g., race V is a chinchilla, of average body weight about 2800 g., and race X is a subline of Castle's small A race, weighing about 2000 g. These races have been bred by matings not wider than parent-offspring, sib-sib, or sib-halfsib for twelve to twenty generations. Race X carries the gene for dwarfism, which when present in double dose is lethal, but in single dose gives rise to rabbits weighing about 1630 g.; dwarfs can usually be distinguished from normal race X members by ear size and other features by age 75 days (Crary & Sawin, 1949), which is the lowest age we are concerned with, but it is possible that a few dwarf gene carriers could be misclassified as normals even after this time and so might appear

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in our population. However, the internal evidence is rather strongly against this: the variability of race X vertebrae from different rabbits but the same part of the spine and the same age group is actually less than that of either of the other races, whereas if a dwarf had been included, one might have expected it to be more.

The age groups used, and the number of rabbits included in each, are given in Table 1. The ages are post-partum, not post-copulatory. The races III and X animals include those reported on previously (Sawin & Dietz, 1950): they were mostly members of the breeding colony sacrificed when no longer productive, or as surplus stock. A few had died from respiratory or intestinal infections of short duration, which would not be presumed to cause changes in the skeleton. The specimens were mostly prepared by skinning, drying of the carcass under refrigeration, and removal of flesh by dermestid beetles. A few were prepared by boiling and removal of flesh. All bones were air dried for some months before measuring.

Table 1. *Number of rabbits of each age group used in study*

	Race		
Age group (days)	III	V	X
a, 75–269	5	9	12
b, 270–399	3	7	8
c, 400–899	7	8	7
d, 900–1440	1	8	6

Vertebral body lengths and breadths were measured; length in the mid-ventral line of the centrum, and breadth at the greatest width of the posterior margin of the centrum. When the epiphyses were still not ossified, as was usually the case in age groups *a*–*c*, the diaphyseal length was measured. Measurements were taken with a trammel bar, and recorded to the nearest mm. in races III and X, and the nearest 0·1 mm. in race V. The measurements of races III and X were done by Mrs Esther Clark, and of race V by Miss Frances Pickle. Only vertebrae 2 to 29 inclusive were measured, and the length of the dens was not included when measuring the axis.

There were six males in the race III material, and six in the race X; all the rest were females. In the rabbit the male is very slightly larger in most skeletal dimensions, but the difference only amounts to about 1 %; in body weight the females are greater (Castle, 1922). In view of the smallness of the skeletal difference, and the fact that the males were evenly distributed amongst the age groups, no separation by sex was made.

RESULTS

The results are presented as a series of tables with illustrative figures. The three races have been compared in pairs, that is V and X, III and V, and III and X. In each comparison an analysis of the total variance presented by the twenty-eight vertebrae, four age groups (or three in the case of race III comparisons) and two races has been made, using the usual technique for a three-way classification. Only one item has been used in each cell, however, and this was the average for the number of rabbits falling within the cell, that is (Table 1) between three and twelve animals (age group *d* for race III has been excluded from the analysis).

The existence of replication within cells, however, makes it possible to estimate the variance of these cell means, and this estimate can be used as the error mean square instead of the triple interaction term, which in fact we particularly want to test for significance. The error variances were obtained by sampling the raw measurements, vertebral locations being chosen at random for each race, subject to the restriction of equal representation of each age group. The calculations are given in Table 2. By comparison of the mean square within-cells figures it will be seen that the three races are quite consistent in variability in length, and races V and X are so in breadth, but race III has a somewhat greater breadth variance. This seems wholly or chiefly due to a large scatter in the breadth figures of the 27th vertebra. For the other two races the breadth scatter at the 27th vertebra seems also to be rather higher than the scatter at other vertebrae, which may be due to some of these vertebrae being sacralized, and others not (race III has about 95 % sacralized, V about 39 % and X about 60 %; Sawin & Hull, 1946). Apart from this, the within-cell variances seem on inspection to be fairly homogeneous both as regards vertebral location and age, and for the error terms they have all been pooled. The variances of the within-cell means are naturally smaller for races V and X than for race III, because of the smaller number of race III rabbits available.

Table 2. *Variances within race, age group and vertebral location (within-cell), and variances of within-cell means ('error' variances)*

Race	Sum of squares	Degrees of freedom	Mean square within-cell	Geometric mean of nos. in cells	Variance of within-cell means
V, length	131·8838	112	1·1775	7·937	0·1484
X, length	138·8125	129	1·0761	7·692	0·1399
III, length	106·7238	108	0·9882	4·564	0·2165
V, breadth	77·0937	112	0·6883	7·937	0·0867
X, breadth	69·4092	129	0·5381	7·692	0·0699
III, breadth	106·8571	98	1·0904	4·564	0·2389

Table 3. *Analysis of variance of length of vertebrae of races V and X, age groups a–d*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Vertebra (location)	2435·6341	27	90·2087*
Race	0·0682	1	0·0682
Age	99·9344	3	33·3115*
Vertebra × race	16·8643	27	0·6246*
Vertebra × age	14·3030	81	0·1766
Race × age	15·7656	3	5·2552*
Vertebra × race × age	1·7294	81	0·0213
	2584·2990	223	
Error	270·6963	241	1·1232 ÷ 7·807 = 0·1439

Tables 3–9 all read the same in method, and the stars against the mean squares indicate significance at the 5 % level. Before considering these tables it may be as well to get clear the interpretations we are to place on a significant result for each of the mean squares.

Vertebra × race × age interaction: different rates of growth in different races in *particular* vertebral regions; that is, the difference in rates applies unequally in different regions.

Vertebra × race interaction: averaging the four age groups, particular vertebral regions differ from one race to the other; that is, the race effect is not equal for all regions of the spine, but is more for some than for others. The averaging of the four age groups is only permissible (or rather, only gives easily interpretable results) if the vertebra-race-age interaction is insignificant.

Vertebra × age interaction: averaging the two races, different regions of the spine grow at different rates during the age span considered. Also only easily interpretable if the vertebra-race-age interaction is insignificant.

Race × age interaction: averaging all the vertebrae (so as to get a general spine effect) the two races grow at different rates. Only easily interpretable if the vertebra-race-age interaction is insignificant.

Vertebra: averaging races and ages, one vertebra differs from another in its dimensions.

Race: averaging all the vertebrae and the ages (which may be misleading if the race-age interaction is significant), one race differs from another in its spine.

Age: averaging the races and all the vertebrae, the spine is still growing over the period considered.

In the present instance we are particularly interested in the vertebra-race-age interaction, and, if this is insignificant, the vertebra-race and race-age interactions.

Table 4. *Analysis of variance of breadth of vertebrae of races V and X, age groups a–d*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Vertebra (location)	1207·6552	27	44·7280*
Race	233·4986	1	233·4986*
Age	5·5776	3	1·8592*
Vertebra × race	9·2562	27	0·3428*
Vertebra × age	5·8026	81	0·0716
Race × age	1·7058	3	0·5686*
Vertebra × race × age	3·4845	81	0·0430
	1466·9805	223	
Error	146·5029	241	0·6079 ÷ 7·807 = 0·0779

It will be seen from the tables that in all instances the vertebra-race-age interaction is insignificant. Neither in length nor in breadth is there any evidence of racial differences in growth rate affecting different regions of the spine differentially, after the age of 75 days. The vertebra-race interaction is significant, however, in every instance, both for length and breadth. Regional differences in vertebrae do exist between these three races, but they are fully established by the age of 75 days, the growth processes causing them having apparently ceased action by this time. It is general growth, not regionally differing growth, that is taking place after this. Not only is there no differential spine growth as between races, but in all except one instance there is none even within the races themselves. The vertebra-age interactions are all insignificant with the exception of that in Table 6, and this one is only barely significant. After 75 days the spine is still growing, in all three races, in length chiefly, but also in breadth, but growing uniformly or nearly so all the way down its length: the backbone is enlarging still, but its differentiation has ceased. It is perhaps rather surprising that the vertebrae are still growing right up to the last age period investigated, that is 900–1440 days or about $2\frac{1}{2}$–4 years. Such adult growth, particularly in length, does not occur in man so far as is known, and it would be very interesting to know the situation in other mammals, but comparable data for most species seem to be lacking. Yale-strain rats reared under good conditions cease growing in length at about 250 days post-partum, which is about 200 days after the time of adolescence (Saxton & Silberberg, 1947).

Race V versus Race X. We may now turn to more particular comparisons between one race and another, taking races V and X, the best documented ones, first. The data are in Tables 3–5 and are illustrated in Figs. 1–4. In Fig. 1 it will be seen that

Table 5. *Analysis of variance of length of vertebrae of races V and X, age groups b–d*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Vertebra (location)	1950·3861	27	72·2236*
Race	4·8348	1	4·8348*
Age	17·1448	2	8·5724*
Vertebra × race	10·7131	27	0·3968*
Vertebra × age	4·5448	54	0·0842
Race × age	0·0201	2	0·0100
Vertebra × race × age	4·6406	54	0·0853
	1992·2843	167	
Error		241	0·1439

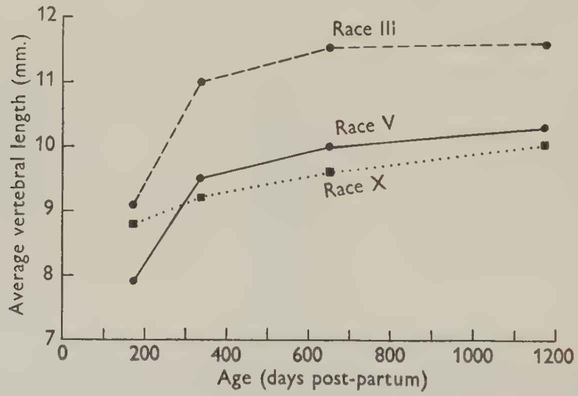

Fig. 1. Comparison of length of vertebral column between three races.

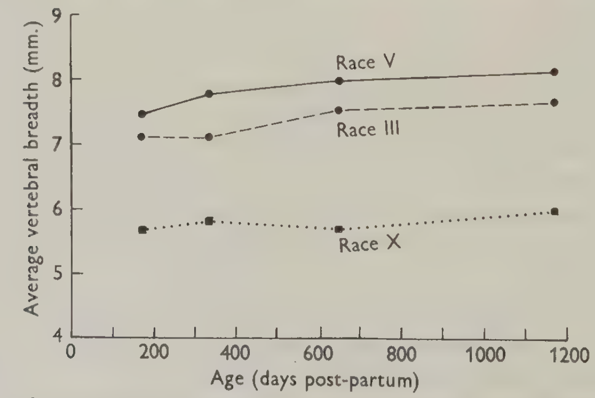

Fig. 2. Comparison of breadth of vertebral column between three races.

race V is longer than race X (the graph is of the average of all vertebral body lengths) at ages over 270 days, but shorter before this. The race-age interaction is significant, and in the figure appears as the lack of parallelism between the V and X lines. The differential growth rate between the races, however, is confined to the first age group considered, occurring before 270 days, and seems to be due only to the broad age categories used; the mean age of race V rabbits in group *a* was 130 days, but for race X, 234 days. For race III it was 132 days. If the group *a* points in Fig. 1 are moved to their correct positions on the time axis, the lines for V and X no longer cross. In Table 5 a second analysis of variance is given of the values for age groups *b–d* only, and here the interaction has disappeared. Also, whereas in Table 3 the race effect was insignificant, due to averaging over age groups *a–d*, in Table 5 it becomes significant; in other words, it is not a chance occurrence that the line for V lies above that for X in Fig. 1 after 270 days. This difference in length is, all the same, small compared to the difference in breadth, which is very striking (Fig. 2), and present at all ages.

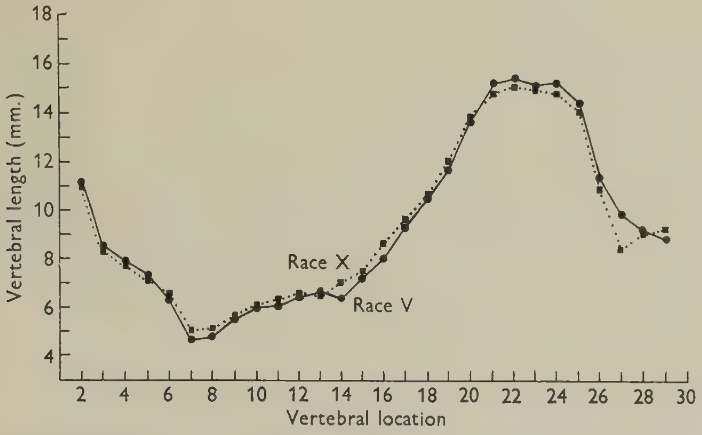

Fig. 3. Comparison of lengths of individual vertebrae between races V and X, age groups *a–d*.

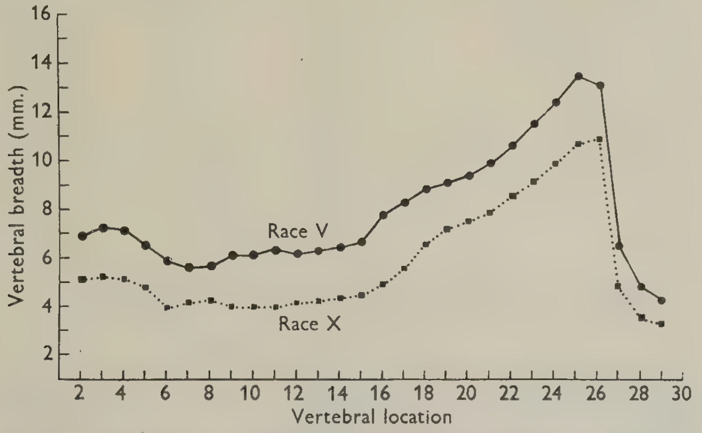

Fig. 4. Comparison of breadths of individual vertebrae between races V and X, age groups *a–d*.

In summary, race V rabbits are much broader than race X, at least in the vertebral skeleton, at all ages over 75 days. They are slightly longer-bodied at ages over 270 days. Both these differences occur equally over the whole spinal column; they are manifestations of general breadth and length controlling forces and not of regionally specific ones.

In addition to the general differences, regional differences exist between V and X, as shown by the significant vertebra-race interactions in Tables 3–5. These differences are shown by the lack of parallelism of the lines in Figs. 3 and 4, where age groups *a–d* have been averaged (since the triple interaction is insignificant). It would seem, from inspection of the figures, and a table of V–X differences for each vertebra, that race X is relatively longer in the posterior thoracic (14–19) and shorter in the lumbar and sacral (21–29, and particularly 27) regions. In breadth, precisely the opposite seems to be true in the thorax, where 14–18 are relatively narrower in X, and in the sacrum, where 27–29 are relatively broader. In the lumbar region 23–25, however, X is narrower as well as shorter. Also in the cervical area 6–8, length and breadth differences go in the same direction, X being broader and longer. Without a very elaborate analysis it is not possible to be sure whether all the differences specifically mentioned, here and below, are statistically significant: probably most of them are, but a few may be chance occurrences.

Table 6. *Analysis of variance of length of vertebrae of races III and V, age groups a–c*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Vertebra (location)	1818·1827	27	67·3401*
Race	81·0029	1	81·0029*
Age	158·8543	2	79·4271*
Vertebra × race	13·8387	27	0·5125*
Vertebra × age	16·3777	54	0·3033*
Race × age	0·3088	2	0·1544
Vertebra × race × age	3·8464	54	0·0712
	2092·4115	167	
Error		220	0·1872

Table 7. *Analysis of variance of breadth of vertebrae of races III and V, age groups a–c*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Vertebra (location)	1106·2964	27	40·7641*
Race	10·3753	1	10·3753*
Age	6·1827	2	3·0913*
Vertebra × race	58·4140	27	2·1635*
Vertebra × age	9·1181	54	0·1689
Race × age	0·8600	2	0·4300*
Vertebra × race × age	8·7185	54	0·1615
	1199·9650	167	
Error		220	0·1443

Race III versus Race V. The analyses of this comparison are given in Tables 6 and 7, and illustrated in Figs. 1, 2, 5 and 6. Race III is the longer at all ages, and there is no significant age-race interaction; the growth rates are the same over the period considered (Fig. 1). In breadth, however, III is less at all ages than V, and here significant interaction does occur. In this race comparison the vertebra-age interaction for length is significant; some differential growth is still occurring. In races III and V the earlier differential growth rates along the column can still be seen, though with difficulty, after 75 days.

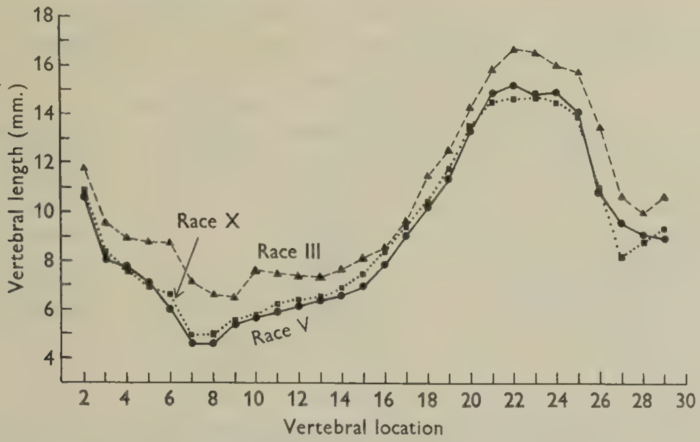

Fig. 5. Comparison of lengths of individual vertebrae between three races, age groups *a–c*.

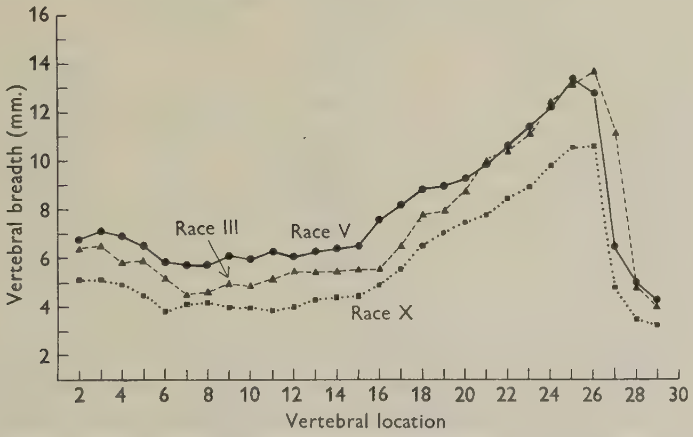

Fig. 6. Comparison of breadths of individual vertebrae between three races, age groups *a–c*.

The vertebra-race interaction is significant both for length and breadth, and from Figs. 5 and 6 and tables of mean differences, it would seem that V is shorter in the 6–8 area and longer in the 13–17 mid-thorax. It is consistently narrower throughout the lumbar and sacral regions, from 20 to 29, and somewhat broader just cephalad of this, from 16 to 19. Possibly the slightly greater broadness of V in the region 7–11 is also significant.

Race III versus Race X. The analyses are given in Tables 8 and 9, and illustrated in Figs. 1, 2, 5 and 6. Race III is both longer and broader at all ages, though both age-race interactions are significant, presumably due in the case of length to the first age group only, as in the V–X comparison.

The vertebra-race interactions are both significant; and the regional differences are not very dissimilar from those between III and V. X is relatively narrower from 20 to 28, and possibly slightly shorter in this region: it is considerably longer in the thorax from 12 to 20, and somewhat broader and shorter in the 6–8 area. Race III has a very broad 27th vertebra relative to both the other races.

Table 8. *Analysis of variance of length of vertebrae of races III and X, age groups a–c*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Vertebra (location)	1729·3148	27	64·0487*
Race	75·9925	1	75·9925*
Age	77·6740	2	38·8370*
Vertebra × race	17·6684	27	0·6544*
Vertebra × age	9·0326	54	0·1673
Race × age	24·1141	2	12·0570*
Vertebra × race × age	2·9998	54	0·0555
	1936·7962	167	
Error		237	0·1809

Table 9. *Analysis of variance of breadth of vertebrae of races III and X, age groups a–c*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Vertebra (location)	1027·2109	27	38·0448*
Race	97·7678	1	97·7678*
Age	1·4629	2	0·7314*
Vertebra × race	54·2474	27	2·0092*
Vertebra × age	9·1148	54	0·1688
Race × age	2·1997	2	1·0998*
Vertebra × race × age	8·3100	54	0·1539
	1200·3135	167	
Error		237	0·1298

DISCUSSION AND CONCLUSIONS

The main conclusions that can be drawn from this study seem to be as follows:

(*a*) The rabbit vertebral column continues to grow in length, and, to a lesser extent, in breadth right up to 2 years or beyond. This is not surprising, as the epiphyses do not close until some time in the third year. Regionally differential growth, however, both within and between races, has ceased before, but probably very little before, the age of 75 days.

(*b*) Races III, V and X differ in three sorts of factors for vertebral body size. These are:

(i) A general vertebral size factor, of which we have no very direct measure, but which is evidently greater in race III than in the other two, and somewhat greater in V than in X.

(ii) A factor for length and breadth of *all* vertebral bodies, that is, a general factor of *linearity of the spine*. Race X rabbits are the most linear, and race V rabbits are least linear, and indeed strikingly broad. This factor may be divisible into two separate and possibly independent biological mechanisms, one influencing length and the other breadth, or both effects may proceed from a single mechanism. This linearity difference may very likely be the equivalent of linearity of physique, or ectomorphy, in the human, which has always been the cornerstone of classifications of human differences in physique.

(iii) Regionally circumscribed factors, also differing in strength from one race to another. These have been the subject of several previous papers in this series (see Peck & Sawin, 1950). Though exact statistical location of the areas in which these factors operate in the mature rabbit has not been made, the regions concerned seem chiefly to be vertebrae 6–8, 13–19, and lumbar (20–26) and sacral (27–29) vertebrae. In the 6–8 area race X is broader (that is, after allowing for factors discussed under (i) and (ii) above) than III or V, and lies between III and V in length, III being the longest, and also the narrowest. In the posterior thoracic area, 13–19 approximately, the differences seem to be mainly of length, with X longest, then V, then III. In both lumbar and sacral regions III is broader than the other races, particularly at vertebra 27: V is broader than X in the lumbar area but narrower in the sacral. Race X appears to be shorter than V throughout the lumbar and sacral areas, but there are no other certain length differences.

This analysis enables us to describe a given race of rabbits, as regards its vertebral column, in some such way as: 'race X are small sized, rather linear rabbits, with an increase in breadth in the posterior cervical region and of length in the posterior thoracic; they are narrow and rather short in the lumbar area, but less narrow at the sacrum'.

It has been known for a long time that general body size is largely determined by genetic factors acting on the body as a whole. This is true both of mammals (Castle, 1922, 1941) and birds (Waters, 1931). The previous studies of this series provided evidence also of regionally circumscribed areas of increased or diminished growth, genetically determined and affecting all tissues in the area concerned (for bibliography see Peck & Sawin, 1950). The present demonstration of racial differences in linearity of the whole spine is clear evidence that *shape* also in animals, as in plants (Sinnott, 1935), is influenced by specific genes. A similar conclusion was reached regarding linearity of build in the human by Brues (1950), and was to be expected following the results of the factor analysis of measurements of rabbits (Wright, 1932) and humans (Burt & Banks, 1947) which yielded results in precise accord with the analysis of variance technique used in the present study. This itself is an interesting biometric point, though not a surprising one; Burt (1947) has described the fundamental similarity between the two techniques.

Practically nothing is known about the genetics of the size, shape and regional factors except that many genes are concerned. In the mouse (Castle, 1941) and rat (Keeler, 1947) several genes affecting body size have also an effect upon coat or eye colour through common physiological mechanisms of an unknown nature. It is usually thought that size genes exist on all or most of the chromosomes.

The mode of action of the regional-area genes has been explored in previous papers in this series, which for the most part, however, dealt with racial differences present at birth, not maturity. As has been pointed out before (Sawin & Dietz, 1950), the presence of an area of accelerated development in newborn animals of a particular race does not necessarily imply that any difference in the area will still be present in mature animals; the other races may develop more slowly, but to the same point. This is true, for example, in the case of adult stature in the human, which is unrelated to the time of the adolescent growth spurt, though during adolescence and before, early developing children are much taller than later developing ones (see Tanner, in press). On the other hand, differences associated with accelerated maturity may persist, and it is believed that certain body contours exemplify this in the human also (see Bayley, 1943; Tanner, 1947). Judged by newborns, race X has accelerated areas at 6–11 and 27–29 (Peck & Sawin, 1950), and in the adult these persist as relative breadth increases in the same areas, except that cervically the localization is restricted to 6–8. Race V newborn has an accelerated area from 13 to 25, but this seems to produce no very noticeable effect in the adult. Race III newborn has accelerated areas at 17–25 and 26–29, and in the adult these areas, more or less, remain broad. It seems likely that these regional growth areas come about through a local increase of tissue early in development in the same way as polydactyly in the cat arises, for example (Danforth, 1947). If so, they may be dependent on relatively few genes. Or it may be that the local effects depend on local increase of sensitivity to the general or to one of the shape influences.

Further advances along these lines would seem to lie in constructing quantitative measures for size, shape and regional factors, and breeding selectivity for extremes in each of them. Such studies might ultimately throw considerable light on the genetics of physique in other species, including man, and thus ultimately on numerous problems of human health and welfare. The importance of genetically controlled animal material in this regard can scarcely be over-emphasized.

SUMMARY

1. The lengths and breadths of the vertebral bodies numbers 2–29 have been measured on the skeletons of three inbred races of rabbits, designated races III, V and X.

2. The age of these rabbits was between 75 days and 1440 days post-partum: in all races growth in length, and to a lesser extent in breadth, proceeds at least up to 2 years of age and perhaps beyond.

3. Pairs of races are compared using analysis of variance technique, with a three-way classification of vertebral location, race and age. The figure in each cell of the analysis is the mean for several rabbits, and its sampling error is given.

4. The conclusion is reached that these races differ in (*a*) general size of all vertebrae, (*b*) linearity of all vertebrae, and (*c*) regional modifications causing local increase in length, breadth, or both. The regions of local growth seem chiefly to be vertebrae 6–8, 13–19, 20–26 and 27–29.

5. No differential growth between regions either in one race or between races occurs after about 75 days post-partum; the racial and regional differences are established before this time.

6. The regional differences described in these adult animals correspond partly to the areas of accelerated growth previously described in the newborn rabbit, but are

partly different. Accelerated development in early life does not necessarily lead to any visible result in the mature animal, though it may do so.

7. The bearing of this result on the genetics of body shape and the classification of physique in man is discussed.

We are greatly indebted to Mrs Esther Clark and Miss Frances Pickle who took the measurements, and to Mr R. H. Whitehouse who did the computing.

REFERENCES

Bayley, N. (1943). Size and body build of adolescents in relation to maturing. *Child Develpm.* **14**, 47–90.

Brues, A. M. (1950). Linkage of body build with sex, age, eye color and freckling. *Amer. J. Human Genet.* **2**, 215–239.

Burt, C. (1947). A comparison of factor analysis and analysis of variance. *Brit. J. Psychol.* (*Stat. Sect.*), **1**, 3–26.

Burt, C. & Banks, C. (1947). A factor analysis of body measurements for British adult males. *Ann. Eugen., Lond.*, **13**, 238–256.

Castle, W. E. (1922). Genetic studies of rabbits and rats. *Publ. Carneg. Instn*, no. 320.

Castle, W. E. (1941). Size inheritance. *Amer. Nat.* **75**, 488–498.

Crary, D. D. & Sawin, P. B. (1949). Morphogenetic studies in the rabbit. VI. Genetic factors influencing the ossification pattern of the limbs. *Genetics*, **34**, 508–523.

Danforth, C. H. (1947). Morphology of the feet in polydactyl cats. *Amer. J. Anat.* **80**, 143–171.

Keeler, C. E. (1947). The effect upon ultimate body size produced by combining certain coat-character genes in the Norway rat. *J. Tenn. Acad. Sci.* **22**, 99–113.

Peck, E. D. & Sawin, P. B. (1950). Morphogenetic studies of the rabbit. VIII. Genetic variations in the sternum as determined by the interaction of general and of regionally specific growth factors. *J. exp. Zool.* **114**, 335–357.

Sawin, P. B. & Dietz, D. B. (1950). Morphogenetic studies of the rabbit. IX. Masking of prenatal growth gradients in adults. In Grüneberg, H. (ed.), *Moderne Biologie*, pp. 215–229. Berlin: Peters.

Sawin, P. B. & Hull, I. B. (1946). Morphogenetic studies of the rabbit. II. Evidence of regionally specific hereditary factors influencing the extent of the lumbar region. *J. Morph.* **78**, 1–26.

Saxton, J. A. & Silberberg, M. (1947). Skeletal growth and ageing in rats receiving complete or restricted diets. *Amer. J. Anat.* **81**, 445–476.

Sinnott, E. D. (1935). Evidence for the existence of genes controlling shape. *Genetics*, **20**, 12–21.

Tanner, J. M. (1947). The morphological level of personality. *Proc. R. Soc. Med.* **40**, 301–308.

Tanner, J. M. (in press). Growth of the human at the time of adolescence. In *Lectures on the Scientific Basis of Medicine*. Vol. I. London: Athlone Press.

Waters, N. F. (1931). Inheritance of body weight in domestic fowl. *Proc. nat. Acad. Sci., Wash.*, **17**, 440–444.

Wright, S. (1932). General, group and special size factors. *Genetics*, **17**, 603–619.

ARTERIO-VENOUS ANASTOMOSES IN THE TONGUE OF THE DOG

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The only author who has reported the presence of arterio-venous anastomoses in the dog's tongue is Brown (1937). She, however, was chiefly interested in the nervous elements of these structures and no adequate account of their morphological features is available. The object of this paper is to show the characteristic form of these direct communications between the arterial and venous systems of the dog's tongue and the appearance of these vessels in different types of preparation.

METHODS

The tongues of more than thirty dogs obtained post-mortem were examined. Only the oral portion of the organ was studied in any detail. Some tongues were not injected and were used solely for histological examination, but in most instances an injection mass was introduced via the lingual arteries. Attempts to inject the tongue from the venous side were unsuccessful, owing to the presence of large numbers of valves in the lingual veins.

Radio-opaque injection masses. Tongues injected with suspensions of bismuth carbonate or barium sulphate were X-rayed both whole and in thick sections to demonstrate the distribution of the major vessels. In spite of the relatively large particle size of these masses, the veins were always filled to a greater or lesser degree.

Neoprene latex. Casts of the lingual blood vessels were made by injection of neoprene latex. The method used in preparing the casts was essentially similar to that described in Trueta, Barclay, Daniel, Franklin & Prichard (1947, pp. 46–7), but after maceration of the tongue in concentrated HCl the cast was placed in 10 % KOH to remove the fat. The injections of neoprene were made at pressures ranging from 200 to 300 mm. Hg. The casts were dissected under water and examined with a stereoscopic binocular microscope.

Berlin blue. This was used in a 2 % solution in distilled water, and proved to be a very satisfactory injection mass in this organ.

Grant's method. In two tongues the walls of the blood vessels were stained by perfusion with Ehrlich's acid haematoxylin by the method of Grant (1930).

Frozen and/or paraffin sections were made of all tongues, other than those injected with neoprene latex, after fixation in 10 % formol saline.

Frozen sections. Sections ranging from 25 to 1000 μ in thickness were cut on the freezing microtome either in the coronal plane or in the planes of the dorsal or ventral surfaces of the tongue. The thinner sections were lightly stained with Ehrlich's acid haematoxylin or alum carmine, the remainder were cleared and mounted unstained.

Paraffin sections. Blocks were embedded in paraffin and cut in one or other of the planes mentioned above, at thicknesses of from 7 to 15μ. In many instances serial sections were made. The majority of sections were stained with Ehrlich's acid haematoxylin and eosin. This proved to be very satisfactory for demonstrating some of the most striking features of the arterio-venous anastomoses, and we preferred these preparations to those stained with Masson's trichrome method. Some sections were stained by Weigert's elastin method, and counterstained with haematoxylin or saffranin. Other sections were stained by van Gieson's method.

For the purpose of demonstrating the general morphology of the arterio-venous anastomoses and their related vessels the use of several injection methods has proved extremely valuable since each method has its own advantages. In neoprene casts, for example, the continuity of the anastomotic channels can be established beyond all doubt by dissection and manipulation under the dissecting microscope. Moreover, any irregularities in the shape of the vessels are faithfully represented in the cast. In thick frozen sections of tongues injected with other masses the communicating channels may be obscured either by overlying vessels or because the plane of section is unsuitable. However, it has been possible to identify with certainty many arterio-venous anastomoses in this type of material, and the fact that other portions of the same tongues could be used for histological examination has proved of great value.

The distinction between artery and vein has presented no difficulty, as each of these vessels has its own characteristic features in the types of preparation studied.

RESULTS

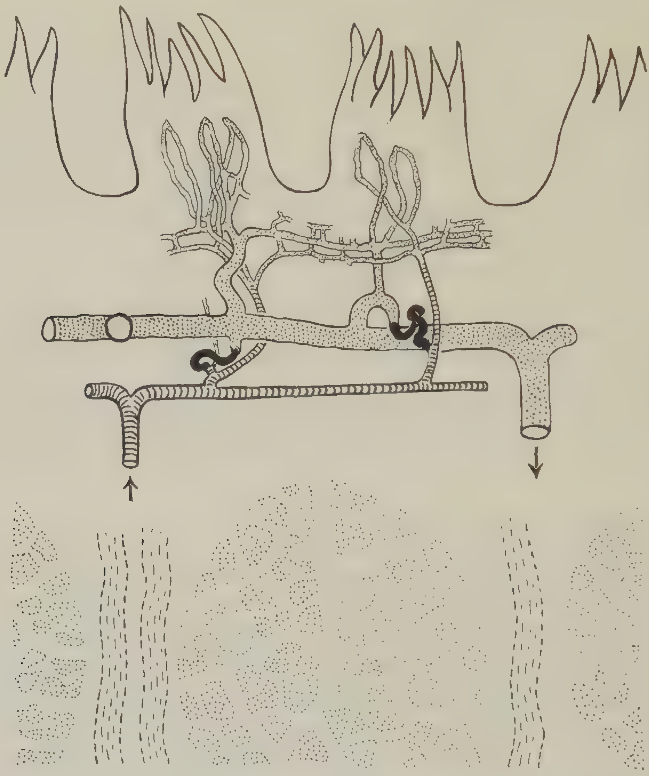
Definition and use of term 'arterio-venous anastomosis'

In describing arterio-venous anastomoses one is faced with a problem of terminology. In general terms an arterio-venous anastomosis can be considered as a channel which permits blood to pass from the arterial to the venous system without traversing a capillary bed. In the tongue, as in certain other organs, the vessel which forms the anastomotic channel can be considered as being composed of three parts, an arterial portion, a venous portion, and a junctional segment. This concept of an arterio-venous anastomosis has arguments in its favour, but the use of these terms presents some difficulty. Since the arterial and venous segments of the anastomosis show no morphological features apart from their continuity with the junctional segment which distinguish them from other small arteries and veins, the determination of the points of origin and termination of the anastomosis must be arbitrary.

Because of this difficulty we have preferred to apply the term arterio-venous anastomosis to the 'junctional segment' alone. This is more convenient and it has this merit, that the term arterio-venous anastomosis is confined to the only segment of the communicating vessel which in virtue of its distinctive features can be identified histologically as such.

Situation of the arterio-venous anastomoses

The intrinsic muscles of the tongue are enclosed, as it were, within a capsule of fibrous tissue, the corium, to which they are attached, and by which they are separated from the epithelium. Running through this layer of fibrous tissue, in a plane parallel to the surface, there are both arteries and veins, the continuations of vessels which, deeper in the tongue, respectively supply and drain the muscles of the organ. The veins lie superficial to the arteries. By the interconnexion of the arteries with one another and by the free anastomosis of the veins, two systems of relatively large vessels are formed in the corium beneath the epithelium, those on the dorsum of the tongue being particularly striking because of their large capacity. The vessels of these arterial and venous systems give off or receive small branches which supply or drain the subepithelial capillary plexus (including, on the dorsum, the capillaries of the papillae), and it is as direct communications between these small arteries and veins, or even between their parent vessels, that the arterio-venous anastomoses are to be found (Text-fig. 1). They are more numerous on the dorsal aspect of the tongue than on the ventral aspect. We have not attempted a detailed count of the arterio-venous anastomoses in the dog's tongue, but an indication of their frequency on the dorsal aspect was given by a paraffin section, cut in the plane of the dorsum, which showed parts of ten separate arterio-venous



Text-fig. 1. Diagram to show the site of arterio-venous anastomoses in the corium of the dorsum of the dog's tongue. The papillae are seen above and the more superficial of the intrinsic muscles of the tongue are indicated below. The arterio-venous anastomoses (solid black) form direct communications between arteries (hatched) and veins (the larger stippled vessels) which supply and drain the subepithelial capillary plexus.

anastomoses in an area of approximately 0·9 sq.mm. It was of interest to note that elsewhere in the same section an equivalent area was found to contain ten papillae. This finding, taken in conjunction with other observations, suggests the possibility that each papilla may have beneath it an arterio-venous anastomosis.

The anastomotic channels are best seen in their entirety in injected preparations when the vessels of the corium are viewed from the deep aspect. Sometimes a good view can be obtained in tissue cut in the coronal plane. Although arterio-venous anastomoses may occasionally be seen adjacent to, or even partially surrounded by, muscle fibres immediately underlying the corium, we have been unable to find these structures amongst the muscles deeper in the tongue.

Morphological features

To appreciate adequately the picture of an arterio-venous anastomosis and its related vessels as seen in histological sections, it is helpful to have examined preparations which show these structures in their entirety and so be familiar with their general morphological characteristics. For such a study, neoprene casts of the vessels of the tongue (Pl. 1, figs. 1, 2) and thick frozen sections of tongues injected with Berlin blue (Pl. 1, figs. 3, 4) have proved extremely valuable, and it is from this type of material that the following brief observations on the general morphological features of the arterio-venous anastomoses and their related vessels have been derived.

The arterio-venous anastomoses form direct communications between arteries and veins which respectively supply and drain the subepithelial capillary plexus, but the precise level at which the connexion is made is variable both on the arterial and the venous sides.

In injected preparations the arterio-venous anastomosis is most commonly seen as a tortuous vessel connecting a small branch of an artery with a small tributary of a vein. The vessels which supply or drain the capillary bed immediately distal to the anastomosis may or may not be demonstrated by the injection mass. The origin of the arterio-venous anastomosis is usually indicated by an abrupt decrease in the lumen of the channel (Pl. 1, figs. 3, 4), and its termination by an increase in the lumen which may be either sudden (Pl. 1, figs. 1, 2) or gradual (Pl. 1, fig. 3). During its course the arterio-venous anastomosis frequently makes an S-bend (Pl. 1, fig. 2), and occasionally two arterio-venous anastomoses, arising from a single artery and opening into one or two veins, are intertwined and form a complex, knot-like structure. Irregularities in the contours of injection masses filling arterio-venous anastomoses show that the lining of the vessel is frequently far from smooth (Pl. 1, figs. 3, 4).

The diameter of the lumen varies, not only from arterio-venous anastomosis to arterio-venous anastomosis, but also in the course of a single arterio-venous anastomosis. Measurements of neoprene casts of these vessels gave diameters of the order of 30–50μ, while cleared preparations of tongues injected with Berlin blue showed diameters ranging from about 10 to 30μ. The lower values obtained in the latter type of material are probably due to the less compact nature of the injection mass and to the fact that this material was subjected to the effects of dehydration. In length the arterio-venous anastomoses were found to range from about 100 to 500μ, the most frequent length being of the order of 200–300μ.

Histological characteristics

One of the most striking features of arterio-venous anastomoses in the dog's tongue is the thickness of their walls (Pl. 2, figs. 5, 7 and 8); this exceeds, by two or three times, that of the average small artery. The thick wall of the vessel is due to the large number of cells which compose the media. These cells consist essentially of two types, smooth muscle cells and 'epithelioid' cells, which are present in variable relative numbers and are irregularly disposed in the vessel wall (see Pls. 2 and 3).

The appearance of the cells varies considerably not only from arterio-venous anastomosis to arterio-venous anastomosis but also from tongue to tongue. Some cells can be readily identified as typical smooth muscle cells, and others as epithelioid cells because of their resemblance to epithelial cells; other cells again, however, appear to be modified versions of one or other of these two types. The typical smooth muscle cells appear to be the exact counterparts of the smooth muscle cells found in the media of muscular arteries. The epithelioid cell, when present in its most highly developed form, is seen to contain a large, pallid nucleus, polyhedral or oval in shape, with a fine stippling of chromatin (Pl. 2, figs. 5, 6; Pl. 3, figs. 10–12). A well-marked nucleolus is not often seen. The borders of the cell are ill defined, and the cytoplasm stains rather poorly with acidophilic dyes.

The wall of the arterio-venous anastomosis contains no internal elastic lamina, although a few elastic fibres may be seen scattered amongst the cells of the media. Endothelial cells are present on the inner surface of the vessel wall, but it has not been possible to establish with certainty whether or not these form a continuous lining to the wall.

The lumen of the arterio-venous anastomosis is frequently very small (Pl. 2, fig. 7), and it is generally irregular in outline (Pl. 2, figs. 5–8). This irregularity, which is an aid in the identification of an arterio-venous anastomosis in sections, is caused by cells of the vessel wall projecting into the lumen (Pl. 3, fig. 9). In specimens in which an injection mass has been used, the arterio-venous anastomosis may show traces of the mass penetrating between the cells of the wall.

Naturally, the precise appearance of an arterio-venous anastomosis in a histological section depends to a great extent on the plane of the section in relation to the vessel and on how much of the anastomosis is shown. The section may include only one part of the vessel (Pl. 2, fig. 7; Pl. 3, fig. 12), or it may show two or more segments of a single arterio-venous anastomosis which, owing to its tortuous course, has been cut at more than one level (Pl. 2, figs. 5, 6 and 8; Pl. 3, figs. 9, 10 and 13). One may see the origin of an arterio-venous anastomosis from its artery, or its termination in a vein, but a section of 7 or 10μ very rarely demonstrates an arterio-venous anastomosis in continuity from artery to vein. Much more frequently an arterio-venous anastomosis and its related vessels are seen as a small group of structures cut in various planes (Pl. 2, fig. 6). In such a complex, capillaries may be seen and a small nerve trunk may also be visible (Pl. 3, fig. 10). The arterio-venous anastomosis itself is enveloped in a sheath of adventitia which often demarcates it from the surrounding fibrous tissue (Pl. 3, fig. 10).

In this investigation we have not made a detailed study of the structure of the vessel wall in the regions of transition between the arterio-venous anastomosis and its afferent and efferent vessels.

In conclusion, one further observation should be mentioned. We found that the histological identification of arterio-venous anastomoses in paraffin sections was easier in some tongues than in others. Fixation of the tissue was in no case unduly delayed so it would appear that this finding was not due merely to differences in the degree of post-mortem changes.

DISCUSSION

It is not our intention here to survey the literature on vessels in various organs which have been described as arterio-venous anastomoses. This has been done by several workers, including Clark (1938), Clara (1927, 1939) and Boyd (1939), each of whom has made a personal study of these structures in one or more organs. Among the tissues, however, which contain vessels that can be accepted as unequivocal arterio-venous anastomoses are the rabbit's ear (Hoyer, 1877; Vastarini-Cresi, 1903; Grant, 1930; Schumacher, 1938), and the human finger and toe (Hoyer, 1877; Grosser, 1902; Vastarini-Cresi, 1902, 1903; Grant & Bland, 1931; Popoff, 1934; Masson, 1937). The anastomoses in these organs, although showing variations in points of detail, have certain characteristic hallmarks. The vessels run a more or less tortuous course; they have a very thick wall, the media containing smooth muscle cells and epithelioid cells in large numbers but in variable relative proportions (according to Clara (1927), and Schumacher (1938), some of the anastomoses in the rabbit's ear are relatively straight and have no epithelioid cells in their walls); in fixed preparations the lumen is usually very small; the vessel has no internal elastic lamina. The arterio-venous anastomoses which are present in the dog's tongue show all these features, and it would appear from our own comparison of these vessels with the arterio-venous anastomoses in the rabbit's ear and in the human finger that the structures are essentially similar in each of these organs. In the tongue of the dog, however, the anastomotic channel is as a rule much simpler in form than it is in the human finger, where the complexity of the unit is one of its most striking features.

As in the case of the arterio-venous anastomoses in the human finger the arterio-venous anastomoses in the dog's tongue are situated in the corium beneath the epithelium. Their position and frequency are such as to suggest that these direct arterio-venous communications, by opening or closing, play an important part in regulating the amount of blood which passes to the subepithelial capillary plexus, including, on the dorsal surface, the capillaries of the papillae. Moreover, the presence of these structures in large numbers suggests that they are devices which, when open, permit a great volume of blood to pass through the superficial parts of the tongue without overburdening the superficial capillary bed. The rich nerve supply of the arterio-venous anastomoses (Brown, 1937) indicates that they are under the control of the nervous system.

The behaviour of arterio-venous anastomoses has been studied in the ears of living rabbits by Grant (1930) and Clark & Clark (1934), who found that these vessels not only responded to various forms of stimulus, but also showed spontaneous rhythmic variations in calibre. Schumacher, who made an extensive anatomical study of the coccygeal body (1908), and also investigated arterio-venous anastomoses in certain other organs, suggested (1938) that the opening and closing of arterio-venous anastomoses which have epithelioid cells is effected by changes in the volume of these cells. He postulated that the lumen was closed by a swelling of the epithelioid cells, due to imbibition of fluid, and that it was opened by a reversal of this process. He also suggested that during their shrinkage the cells might liberate a substance resembling acetylcholine, and that thus the arterio-venous anastomoses would have more than a purely local circulatory effect.

Many theories have been advanced as to the function of arterio-venous anastomoses in general (see, for example, Clark, 1938, and Clara, 1939). It has been suggested that they regulate body temperature, blood pressure, and hydration of the tissues, and also that they provide a mechanism for improving the venous return to the heart. The fact that on the whole they occur more commonly in the superficial and peripheral parts of the body rather than in deeper sites has been adduced as evidence for some of these theories. That the arterio-venous anastomoses in the rabbit's ear respond to temperature changes has been shown in the living animal by Grant (1930), Grant, Bland & Camp (1932) and Clark & Clark (1934). As a result of other studies, Grant & Bland (1931) concluded that the arterio-venous anastomoses in the human finger were the vessels chiefly responsible for the increased blood flow which they observed as a reaction to cooling.

Brown (1937) has suggested that the arterio-venous anastomoses in the dog's tongue are concerned with the elimination of heat, a theory which is attractive since the dog has few sweat glands in the skin and protrudes its tongue when panting. On the other hand, we have recently discovered that the tongues of sheep and goats have arterio-venous anastomoses of similar type and in the same situation, an observation which is of interest in view of the different habits of these animals. In none of these animals are salivary glands present in the corium of the anterior portion of the tongue, so the arterio-venous anastomoses situated here cannot be directly concerned with glandular secretion.

SUMMARY

The arterio-venous anastomoses in the corium of the dog's tongue have been studied in injected preparations and histologically. These vessels form direct communications between arteries and veins which respectively supply and drain the subepithelial capillary plexus. They run a more or less tortuous course. They have a very thick wall, the media of which contains smooth muscle cells and epithelioid cells in large numbers but in variable relative proportions. The vessels have no internal elastic lamina, and in fixed preparations the lumen is usually very small. Their situation and great numbers, particularly on the dorsum, suggest that these vessels when open allow a large volume of blood to pass through the superficial parts of the tongue without overburdening the subepithelial capillary bed.

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REFERENCES

Boyd, J. D. (1939). Arterio-venous anastomoses. *Lond. Hosp. Gaz.* **42**, ii–viii.

Brown, Margaret E. (1937). The occurrence of arterio-venous anastomoses in the tongue of the dog. *Anat. Rec.* **69**, 287–292.

Clara, M. (1927). Die arterio-venösen Anastomosen der Vögel und Säugetiere. *Ergebn. Anat. EntwGesch.* **27**, 246–301.

Clara, M. (1939). *Die arterio-venösen Anastomosen.* Leipzig: J. A. Barth Verlag.

Clark, E. R. (1938). Arterio-venous anastomoses. *Physiol. Rev.* **18**, 229–247.

Clark, E. R. & Clark, E. L. (1934). Observations on living arterio-venous anastomoses as seen in transparent chambers introduced into the rabbit's ear. *Amer. J. Anat.* **54**, 229–286.

Grant, R. T. (1930). Observations on direct communications between arteries and veins in the rabbit's ear. *Heart*, **15**, 281–303.

Grant, R. T. & Bland, E. F. (1931). Observations on arterio venous anastomoses in human skin and in the bird's foot with special reference to the reaction to cold. *Heart*, **15**, 385–411.

Grant, R. T., Bland, E. F. & Camp, P. D. (1932). Observations on the vessels and nerves of the rabbit's ear with special reference to the reaction to cold. *Heart*, **16**, 69–101.

Grosser, O. (1902). Ueber arterio-venöse Anastomosen an den Extremitätenenden beim Menschen und den krallentragenden Säugethieren. *Arch. mikr. Anat.* **60**, 191–216.

Hoyer, H. (1877). Ueber unmittelbare Einmundung kleinster Arterien in Gefässäste venösen Charakters. *Arch. mikr. Anat.* **13**, 603–644.

Masson, P. (1937). *Histophysiolgie.* IV: *Les glomus neuro-vasculaires.* Ed. by A. Policard. Paris: Hermann et Cie.

Popoff, N. W. (1934). The digital vascular system. *Arch. Path.* (*Lab. Med.*), **18**, 295–330.

Schumacher, S. (1908). Über das Glomus coccygeum des Menschen und die Glomeruli caudales der Säugetiere. *Arch. mikr. Anat.* **71**, 58–115.

Schumacher, S. (1938). Ueber die Bedeutung der arteriovenösen Anastomosen und der epitheloiden Muskelzellen (Quellzellen). *Z. mikr.-anat. Forsch.* **43**, 107–130.

Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J. & Prichard, M. M. L. (1947). *Studies of the Renal Circulation.* Oxford: Blackwell Scientific Publications.

Vastarini-Cresi, G. (1902). Communicazioni dirette tra le arterie e le vene (anastomosi artero-venose). Nota preliminare. *Monit. zool. ital.* **13**, 136–142.

Vastarini-Cresi, G. (1903). *Le anastomosi artero-venose nell' uomo e nei mammiferi. Studio anatomo-istologico.* Napoli: F. Sangiovanni. (Quoted by Clara, 1939.)

EXPLANATION OF PLATES

Plate 1

Figs. 1 and 2. Neoprene latex casts of vessels in corium of dorsal surface of dog's tongue, viewed from the deep aspect. The arteries (*A*) and the veins (*V*) form a capacious vascular bed lying beneath the epithelium, the capillaries of which they supply and drain. The two systems are connected by numerous arterio-venous anastomoses, some of which are here indicated by arrows. Note that in these examples the calibre of the vessel increases abruptly where the arterio-venous anastomosis joins the vein. In fig. 2, close to the origin of the arterio-venous anastomosis, an arterio-arterial junction is seen, this being indicated by a marked bend and a narrowing of the continuous vessel.

Fig. 3. Dog's tongue injected with Berlin blue. Arterio-venous anastomosis (at arrow) in the corium, as seen in a thick frozen section cut in the plane of the dorsum. Note the tortuosity of the anastomosis and the narrow irregular lumen. *A*, artery; *V*, vein.

Fig. 4. Frozen section of similar preparation, lightly stained with alum carmine, showing an arterio-venous anastomosis (at arrow). Note the narrow irregular lumen and also the thick wall of the anastomosing vessel, the outline of which can just be seen along its proximal portion. *A*, artery; *V*, vein.

Plate 2

Fig. 5. An arterio-venous anastomosis, cut at two levels, is seen lying above one of the large arteries of the corium (*A*). Note the irregular outline of the injection mass in the lumen of the anastomosis, and the large pale nuclei of the epithelioid cells in the thick wall of the vessel. Injected preparation: haematoxylin and eosin.

Fig. 6. Group of vessels in corium of dog's tongue. To the left and above are three segments of an arterio-venous anastomosis cut at different levels. To the right of and below these are two small arteries and a capillary. (The identity of the vessels was confirmed by study of the adjacent section which was stained for elastic fibres, an internal elastic lamina being absent in arterio-venous anastomoses.) Injected preparation: haematoxylin and eosin.

Fig. 7. An arterio-venous anastomosis is seen cut in cross-section and lying between an artery (*A*) and a vein (*V*). The appearance of the anastomosis, with its thick, cellular wall and narrow lumen, is typical. Haematoxylin and eosin.

Fig. 8. Arterio-venous anastomosis cut at two levels. Note the modified smooth muscle cells and epithelioid cells irregularly disposed in the wall of the vessel. Haematoxylin and eosin.

PLATE 3

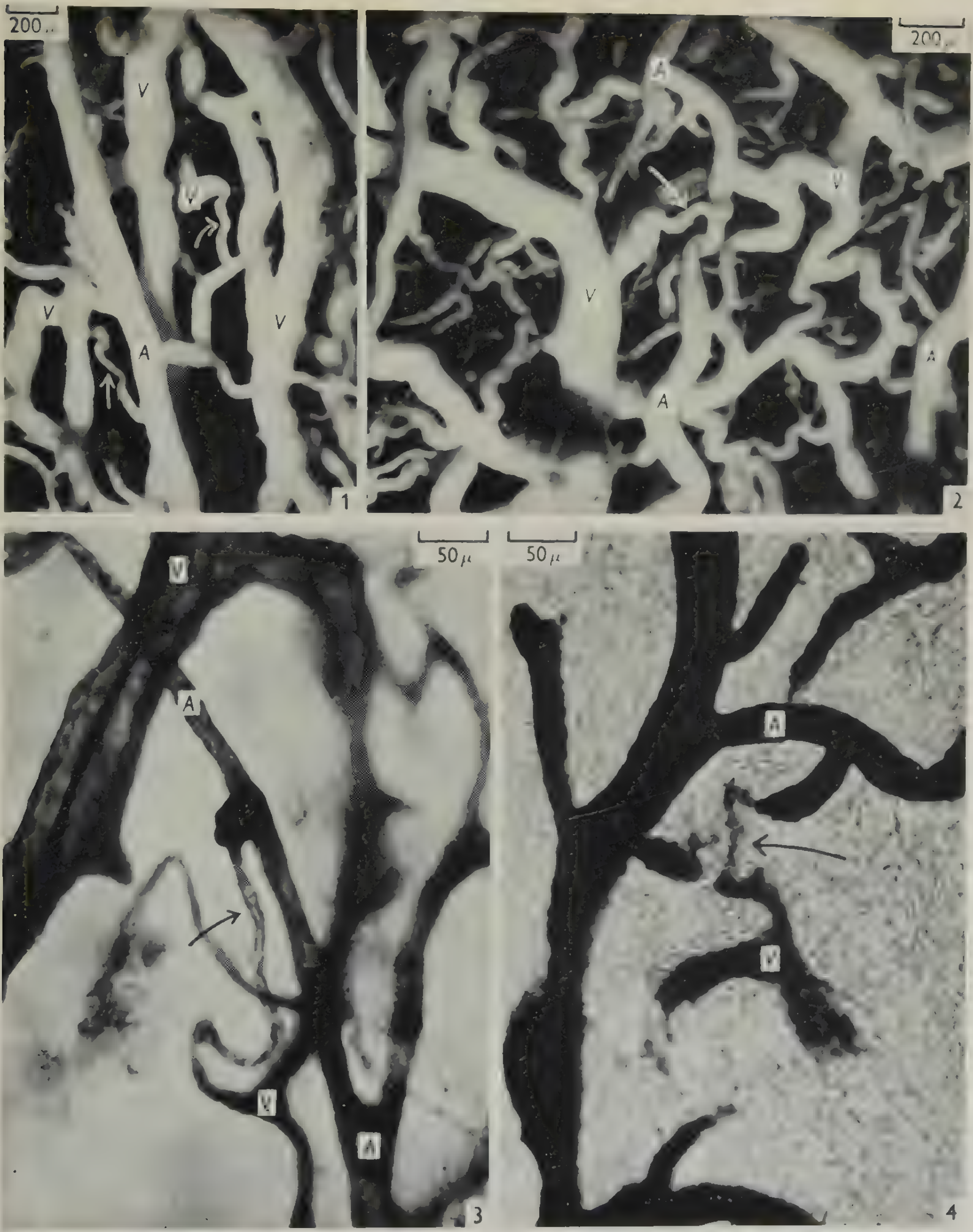
Fig. 9. Part of an arterio-venous anastomosis, the venous end of which is seen below. An artery (containing injection mass) is seen in cross-section on the right. In the portion of the anastomosis seen above, note the cells projecting into the lumen of the vessel, giving the outline of the latter its characteristic irregularity. In this instance most of the cells of the vessel wall appear to be of modified smooth muscle type. Injected preparation: haematoxylin and eosin.

Fig. 10. Arterio-venous anastomosis seen as a compact unit, well demarcated from the surrounding tissue. The section shows two segments of the anastomosis cut transversely, and also part of the wall of the intermediate segment cut obliquely. Epithelioid cells are present in the wall of the vessel. A small nerve trunk is seen on the left. Injected preparation: haematoxylin and eosin.

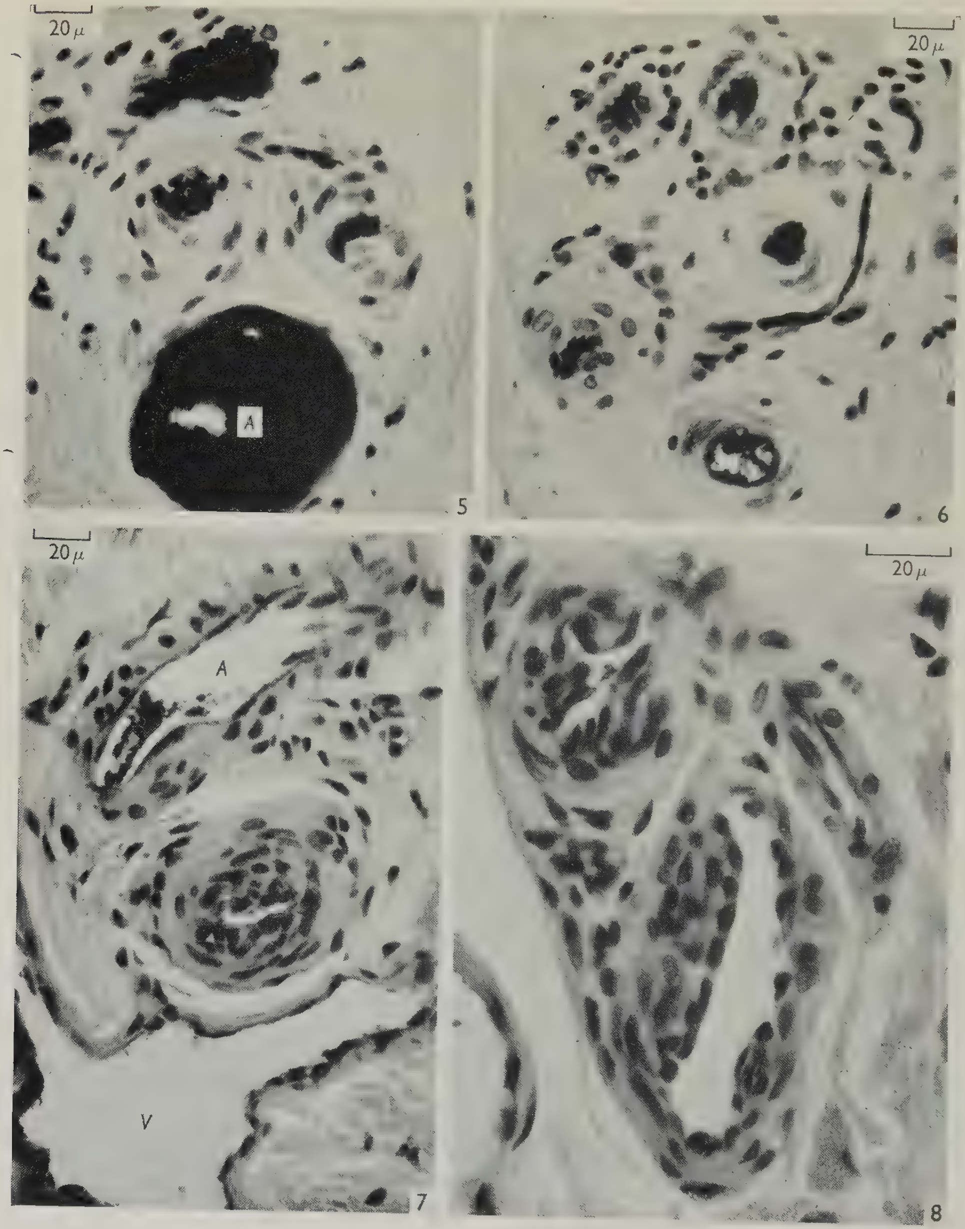
Fig. 11. Part of an arterio-venous anastomosis seen in longitudinal section. Note the marked bend of the vessel and the many large pallid nuclei of the epithelioid cells. Injected preparation: haematoxylin and eosin.

Fig. 12. Oblique section through an arterio-venous anastomosis. Many of the cells in the vessel wall are of epithelioid type, with large pale nuclei. Haematoxylin and eosin.

Fig. 13. Arterio-venous anastomosis cut obliquely, with its venous end below on the left, and a small artery on the right. A unit such as this is frequently seen (cf. fig. 9). Both epithelioid and modified smooth muscle cells are seen in the wall of the anastomosis. Haematoxylin and eosin.



PRICHARD AND DANIEL—ARTERIO-VENOUS ANASTOMOSES IN THE TONGUE OF THE DOG



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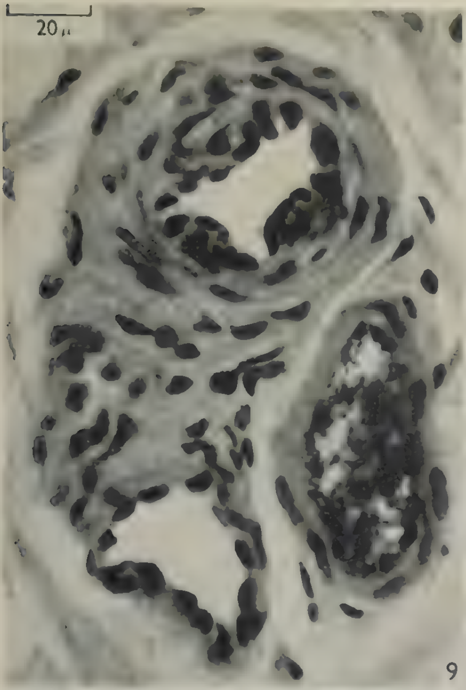

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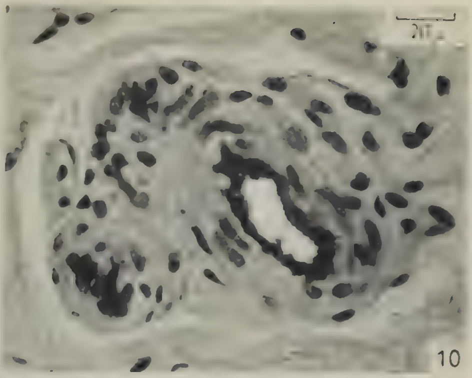

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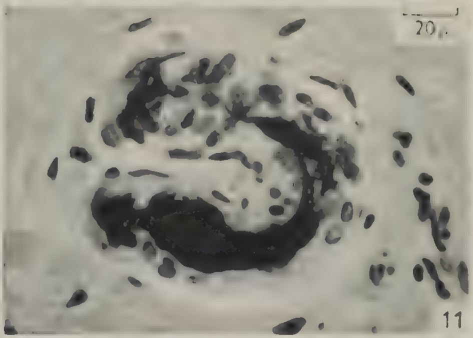

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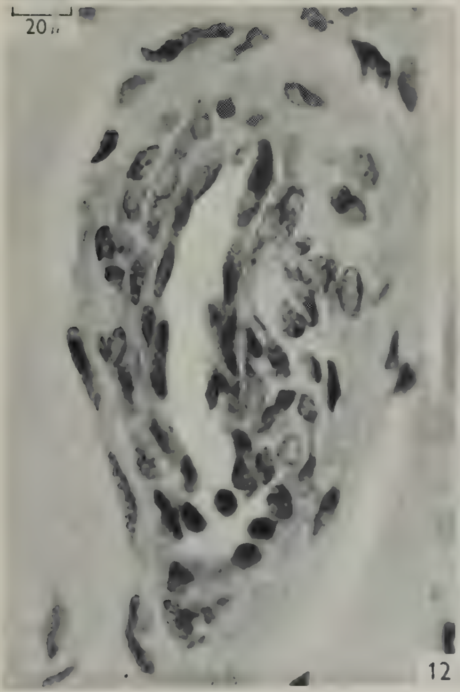

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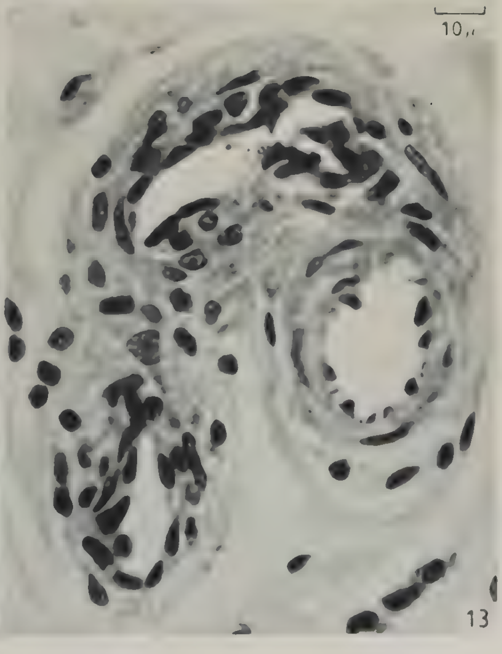

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PRICHARD AND DANIEL—ARTERIO-VENOUS ANASTOMOSES IN THE TONGUE OF THE DOG

THE INSERTIONS OF THE FLEXORES POLLICIS LONGUS ET DIGITORUM PROFUNDUS

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The progressive development of the faculties of manual palpation and prehension was a most significant factor in human evolution. The volar aspect of the digits of the hand is therefore of considerable interest, yet there are features of its topographic anatomy which have passed unnoticed.

Dissection of a hundred fingers and a hundred thumbs from adult human cadavers has revealed fundamental differences, probably of morphological significance, between the distal insertions of the flexor pollicis longus and flexor digitorum profundus tendons. These have been compared and correlated with relevant findings in forty human little toes and a wide series of animal dissections. The synovial flexor sheaths of fourteen adult human fingers and thumbs have been injected and ninety terminal phalanges have been examined.

DIFFERENCES BETWEEN THE INSERTIONS OF THE TWO TENDONS

In man each flexor digitorum profundus tendon is, to quote Albinus (1749) '...in a manner split longitudinally and appears as if formed of two conjoined together'. Each tendon possesses a distinct longitudinal ventral median furrow and a shallower dorsal one. The volar groove can usually be distinguished opposite the metacarpophalangeal joint. Though only narrow here, it becomes much more evident distal to the bifurcation of the flexor digitorum sublimis, and is deepest opposite the middle phalanx, where it sometimes almost divides the tendon into two halves.

This mid-line division was noted by Winslow (1733), Boyer (1815), Bichat (1819), Cloquet (1822), Meckel (1825) and Poirier & Charpy (1901), but is not described in standard English anatomical works.

The superficial tendon fibres of a flexor digitorum profundus tendon, when traced distally from the level of the bifurcation of the corresponding sublimis tendon, gradually diverge from this mid-line furrow towards the tendon margins. Opposite the terminal interphalangeal joint the volar fibres diverge more acutely to insertions on each side of the ventral surface of the base of the distal phalanx, so exposing the deeper fibres which are attached more distally and centrally on the volar aspect of the shaft of this bone (Pl. 1, fig. 1).

In the thumb the flexor pollicis longus tendon also possesses a ventral groove from which fibres diverge. The superficial fibres of this tendon at the level of the terminal joint pass distally to insertions on the shaft of the terminal phalanx. The deepest fibres form *two discrete lateral tendon fasciculi* which diverge to be inserted on each side of the volar surface of the base of this bone (Pl. 1, fig. 2). Sometimes, one of these fasciculi receives an accession of a few fibres from the margin of the tendon (Pl. 1, fig. 3). Transverse 100μ sections of a flexor pollicis longus tendon emphasize the discrete nature and predominantly deep origin of the lateral fasciculi (Pl. 1, fig. 4).

The flexor pollicis longus tendon of *Macacus rhesus* shows similar lateral fasciculi but these are ill-defined or absent in several other primates examined and are lacking in the lower animals which have been dissected.

A ligamentous band extends on each side between the lateral aspects of the base of the phalanx and the proximally projecting tips of the tuberositas unguicularis and protects the vessels and nerves which pass dorsally from the pulp to the nail bed. A few of the laterally inserted fibres of the long flexor tendon both in the finger and the thumb are continued into this ligament (Pl. 1, figs. 1–3). Some of the centrally inserted fibres in the thumb, and to a lesser extent in the fingers, are sometimes relayed from the shaft to the base of the tuberositas unguicularis (Pl. 1, fig. 3).

Briefly stated, when traced distally from the level of the terminal joint, the most *superficial* tendon fibres in the finger pass to lateral insertions, and in the thumb they go to more central and distal insertions. The *deep* tendon fibres in the finger have central distal insertions, and in the thumb they possess lateral basal insertions.

THE TERMINAL PHALANGES

There are differences between the bony roughenings for tendon insertions in the thumb and the fingers; these roughenings are coloured black in Pl. 1, figs. 5 and 7.

In the thumb the main central insertion and the two lateral fasciculi are sometimes associated with separate bony tuberosities (Pl. 1, fig. 5*a*), but more commonly the latter are united by two slender ridges (Pl. 1, fig. 5*b*), often undermined by a small vessel. Radiographs of twenty-five living subjects showed that during flexion of the terminal phalanx, the mid-line volar sesamoid (present in 72 %) comes into apposition with the base of the phalanx, in the concavity of this U-shaped insertion (Pl. 1, fig. 6).

In the terminal phalanges of fingers the roughening for flexor tendon attachment is limited proximally by a transverse bony ridge (Pl. 1, fig. 7).

The insertions of these flexor tendons is primarily diaphysial, extending more than half way from the articular margin to the base of the tuberositas unguicularis. In the adult, and also in the 3½ months' foetus, the flexor tendons of the terminal phalanges reach much more distally than the corresponding extensor tendons, as is well demonstrated by sagittal sections (Wilkinson, 1951).

THE DIGITAL SYNOVIAL FLEXOR SHEATHS

Papers on the digital synovial sheaths by Fourcroy (1785), Chemin (1896), Whittaker (1907), Lucien (1910) and Retterer (1896, 1918*a*) and the anatomical atlases of Spalteholz (1901), Toldt (1944), Grant (1947) and Sobotta (1948) fail to record certain differences which exist between the distal termination of the polliceal flexor sheath and that in other digits. Poirier (1901) reported that the visceral layer of the sheath is reflected from the tendon distally in a 'preputial' manner, forming a 'simple annular cul-de-sac', a view commonly held.

There are technical difficulties in filling the cavities of these sheaths. These may be overcome in the following manner. A digit is amputated at the metacarpophalangeal joint and the synovial sheath opened. The digit is then flexed so that the cut end of the flexor tendon extrudes from the sheath. The tendon is divided flush with the amputated stump, so that when the digit is again extended, the tendon retreats into its canal, and a small pocket is formed in the proximal portion of the sheath. A glass cannula is inserted into this pocket and sealed in position with gelatine. The digit is then placed within a large test-tube, which has been half-filled with warm gelatine. More gelatine is added until the digit and the lower half of the cannula are immersed. When the gelatine sets it forms a perfect seal between the synovial sheath and the cannula and also maintains the latter in position. A small quantity of 10 % colloidal silver iodide is dropped into the cannula, after which the test-tube with its contents is centrifuged at 900 r.p.m. for 15 min. The colloidal particles having now been forced to the distal tip of the synovial sheath, supernatant fluid in the cannula is replaced with silver iodide and the centrifuging continued for half an hour. This process is repeated until fluoroscopic screening indicates that the sheath is filled. Though a somewhat elaborate procedure, this is the only method, of many tried, which portrays the details of the distal portion of the cavity of the synovial sheath.

The synovial sheath of the flexor pollicis longus

The terminal part of the lumen of this sheath has a small cul-de-sac on either side which possesses a slight dorsal inclination (Pl. 2, fig. 8). Dissection of the injected sheath indicates that these lateral diverticula lie on the volar surface of the lateral fasciculi of the flexor pollicis tendon. They are demarcated by fusion of the visceral and parietal synovial layers along the sulci which separate the central and lateral parts of the tendon at its insertion. When the median furrow in the ventral surface of the tendon is well marked, the parietal layer is carried into it prematurely, and by blending with the visceral layer creates a central cleft in the distal tip of the sheath.

The synovial sheath of the flexor digitorum profundus

The distal portion of the cavity of this sheath is more rounded than in the thumb (Pl. 2, fig. 9). There is no mid-line cleft in the tip of the sheath, nor are lateral diverticula present in its terminal extremity. Where the visceral synovial membrane is reflected from the tendons of the *flexor digitorum sublimis* as the latter pass to their insertions, two small diverticula are seen, comparable in shape and dorsal inclination to those noted in the thumb.

Thus a careful examination of the synovial sheaths in the thumb and the fingers emphasizes the discrete nature of the lateral tendon fasciculi of the flexor pollicis longus.

A SUGGESTED EXPLANATION OF THESE DIFFERENCES

Why are there such striking differences in the mode of insertion of these two long flexor tendons? To answer this question attention is directed to the lateral tendon fasciculi of the flexor pollicis longus. These probably represent rudiments of a flexor perforatus tendon. The evidence for this view will be discussed under four headings: (1) the morphology of the terminal phalanx; (2) flexor tendons in the toes; (3) digital flexor tendons in lower animals; (4) polliceal 'perforatus' tendons in man.

(1) *The morphology of the terminal phalanx*

The pollex possesses three bones distal to the carpus; all other manual digits have four. The problem implicit in this statement underlies a controversy two thousand years old. It is unnecessary to recount every opinion expressed about the nature of the first metacarpal bone as these have been well summarized by Welcker (1884), Krause (1896), Wakeley (1924) and Jones (1949).

The first metacarpal has been held to represent a phalanx, but there is now fairly general agreement that this is not so. It is of historical interest to note that Galen (A.D. 131–200) and Vesalius (1555) maintained that the four ulnar metacarpal bones articulate proximally by synarthroses and distally by diarthroses, whereas the first 'metacarpal' possesses both *proximal* and distal diarthroses and is thus to be considered a phalanx. The foundation for this theory, therefore, rests on what is now patently a fallacy. The basal position of the epiphysis does not always distinguish the first from other metacarpals in animals or even in man. Moreover, the relative mobility of the first metacarpal bone does not affect its homology with the first metatarsal, though the two bones are equally mobile in the anthropoid ape.

The general process of phalangeal reduction has been studied in foetal cetacea by Kükenthal (1888), who stressed that the sequence of reduction comprises a fusion of terminal with subterminal phalanges. It is therefore interesting to recall Gräfenberg's assertion (1905), that the diaphysial ossification of human terminal phalanges takes place from two centres which soon fuse, thus recapitulating an ancient phylogenetic reduction. Gräfenberg also showed that the terminal phalanx of the thumb is longer than the other terminal phalanges at all stages of development.

There are many records of human tri-phalangeal thumbs (Windle, 1891; Salzer, 1897; Pfitzner, 1898; Joachimsthal, 1898, 1900; Rieder, 1900; Jurčić, 1906; Geelvink, 1913; Kristjansen, 1926; Lapidus & Guidotti, 1943). In these cases the terminal phalanx is usually shortened, and an accessory element (middle phalanx) is present between this and the basal phalanx. This suggests that the terminal polliceal phalanx represents two fused phalanges, an inference, however, which is open to some criticism, for even the palaeozoic stegocephali had only two polliceal phalanges (Windle, 1891).

It has been shown that the lateral fasciculi of the flexor pollicis longus are inserted into the base of the terminal phalanx. It would be very interesting to know whether these tendon bundles are attached to the small middle phalanx in the abnormal three-membered thumb of man. The accounts of tri-phalangeal thumbs deal only with external and radiological appearances and no account of a dissection of this abnormality has been discovered.

Having studied the process of marginal phalangeal reduction by bony fusion between the middle and terminal phalanges of the little toe, Pfitzner (1900) concluded that the ungual phalanx of the thumb is similarly a compound structure derived from two phalanges. Jones (1949) stated 'I know no fact derived from any study of normal or abnormal anatomy or from paleontology that contradicts this finding'.

(2) *Flexor tendons in the toes*

The arrangement of the tendon fibres of the flexor digitorum longus at its distal insertion in the second, third and fourth toes closely resembles that seen in its manual counterpart, the flexor digitorum profundus. In the case of the marginal digits, however, there are certain differences.

The great toe

The importance of the human great toe in bipedal locomotion is reflected in the commonest pedal digital formula. The size of the flexor hallucis longus tendon in man necessitates extensive distal attachments, and these appear to have undergone some modification. The uniformity of tendon fibre arrangement found in the insertion of the corresponding polliceal flexor tendon is lacking in the hallux, and comparable discrete lateral tendon fasciculi are rarely distinguishable. The changes of structure in this toe in response to alteration of function will be dealt with in a further communication.

The little toe

Skeletal changes in this toe have been shown by Pfitzner (1890, 1896) and others to shed light on the nature of the osseous structure of the thumb. This writer recorded (1896) fusion of the middle and terminal phalanges in 37 % of 838 little toes, and stated (1890) that in toes with two phalanges the perforatus tendon was *always* rudimentary or absent. In the present series of forty little toes fusion of the middle and terminal phalanges is present in 25 %, and in half of the latter the reduction of the middle phalanx co-exists with a reduction of its associated short flexor tendon as a separate entity. In each of four of these toes the perforans tendon was found to provide a fasciculus, histologically proved to be of a tendinous nature, to a fused middle phalanx (Pl. 2, fig. 10). In one toe with phalangeal fusion the perforans and perforatus tendons unite immediately prior to their insertions (Pl. 2, fig. 11). In only one toe with three phalanges is there an extensive fusion of tendons, the perforans tendon providing two fasciculi to a separate middle phalanx (Pl. 2, fig. 12).

If the inter-related normal perforans and perforatus tendons of the little toe are compared with the insertion of the flexor pollicis longus (Pl. 2, fig. 13), a marked resemblance is noted, the perforatus in the little toe corresponding to the lateral tendon fasciculi in the thumb.

Since the base of the terminal polliceal phalanx probably contains elements of a fused middle phalanx, these observations on little toes suggest that the *polliceal lateral fasciculi represent rudiments of flexor perforatus tendons.*

(3) *Digital flexor tendons in lower animals*

In Amphibia and Reptilia, short muscles of palmar origin provide digital tendons which are often perforated by the flexor tendon of the terminal phalanx. In digits with more than three phalanges, more than one pair of perforatus tendons are sometimes present. No example has been found of true polliceal perforatus tendons.

In Mammalia below the order Primates it is necessary to distinguish between the flexor tendons in the digits and their proximal muscular connexions. From one to three different muscles may be attached to a *digital* perforatus tendon. The muscular attachments of these tendons in twelve animals are shown diagrammatically in Table 1.

(i) *The flexor digitorum sublimis.* This muscle may arise from the ulnar-sided epicondyle of the humerus (e.g. *Felis domestica*, *Mephitis*), or it may take origin from the musculo-tendinous stratum which provides flexor tendons to the terminal phalanges (e.g. *Canis familiaris* and the marsupials).

(ii) *The palmaris longus.* The most superficial flexor muscle of the antebrachium is, to a variable degree, divided into discrete digital tendons in the manus. The distinctness of these tendons strongly suggests an important function in digital flexion and they are sometimes misnamed accordingly. Thus in *Canis familiaris* the large muscle referred to as the 'flexor digitorum sublimis' by Bradley (1912) is in fact the palmaris longus, and the small deep muscles ('palmaris accessorius'—Sisson, 1910) which join its tendons are strictly comparable to the flexor digitorum sublimis in marsupials. Thus Huxley (1871) and Windle & Parsons (1897) were incorrect in assuming that the palmaris longus is absent in this animal.

Table 1. *Muscles inserted into digital perforatus tendons*

Name	1	2	3	4	5	Name	1	2	3	4	5
Macropus bicolor						*Mephitis mephitis*	▨	▨	▨	▨	▨
		■	■	■				■	■	■	
					░						
M. rufogriseus fruticus						*Nasua nasua*		▨	▨	▨	▨
		■	■	■				■	■	■	
					░						░
Metachirus opossum						*Oryctolagus cuniculus*					
		■	■	■				■	■	■	■
					░						
Erinaceus europaeus		▨	▨	▨	▨	*Cavia porcellus*					
		■	■	■				■	■	■	
											░
Felis domestica		▨	▨	▨	▨	*Thyronomys sp.*					
		■	■	■				■	■	■	
				░	░						░
Canis familiaris		▨	▨	▨	▨	*Meles meles*		▨	▨	▨	
		■	■	■					■	■	
											░

KEY. Palmaris longus ▨
Flexor digitorum sublimis ■
Flexor brevis digitorum manus ░

(iii) *Flexor brevis digitorum manus.* A short muscle, usually of palmar origin, is sometimes inserted into digital perforatus tendons. In *Felis domestica* this muscle arises from the lower portion of the palmaris longus tendon. As Haines (1950) also noted, the perforatus tendons of the fourth digit of this animal receives contributions from three sources (See Table 1).

The tendon ring

Integration of these various contractile elements is brought about by their attachment to a fibrous or fibrocartilaginous ring which encircles each profundus tendon at the level of the metacarpo-phalangeal joint. The digital perforatus tendons extend distally from this ring, and palmar tendons of diverse muscular origin are attached to it proximally. Though Windle & Parsons (1897) referred to the widespread presence of tendon rings in insectivores, rodents and carnivores, accounts of such rings are often lacking in the descriptions of these orders by other writers. In *Canis familiaris* the tendon rings are particularly well developed and constitute the fibrocartilaginous tunnel described by Douglas (1763) and Retterer (1918*b*). Parsons (1898) noted the occasional presence of this ring in man.

Palmar tendons of a flexor perforatus complex are often not in line with the digit they supply. Tendon rings ensure apposition and alignment of digital perforatus and perforans tendons and also protect them at the level of the metacarpo-phalangeal joint. These rings are not present in the marsupials examined, in which only a single superficial flexor tendon passes to each digit.

Table 2. *Polliceal 'sublimis' tendons*

Author	Animal	Muscle supplying the tendons	Digital insertion
Humphry (1869)	*Manis*	Flexor dig. sublimis	1st phalanx
Humphry (1872)	*Uromastix spinipes*	Flexor dig. sublimis	1st phalanx
Mivart (1881)	*Felis domestica*	Flexor dig. sublimis	Ulnar side of pollex
Dobson (1882)	*Erinaceus algirus*	Palmaris longus	?
	E. jerdoni	Flexor dig. sublimis	?
Shepherd (1883)	*Ursus americanus*	Flexor dig. sublimis	2nd phalanx
Bardeleben (1894)	*Hyrax brucei*	Flexor brevis superficialis	?
	Paradoxurus	Flexor dig. sublimis	2nd phalanx
Windle & Parsons (1897)	*Procyon lotor*	Flexor dig. sublimis	?
	Nasua	Flexor dig. sublimis	?
	Ursus americanus	Flexor dig. sublimis	?
	Felis catus	Flexor dig. sublimis	?
Frazer (1907)	*F. domestica*	Flexor dig. sublimis	Ulnar side of pollex
Howell (1932)	*Dipodomys*	Flexor dig. sublimis	?

Polliceal 'perforatus' tendons

There are many descriptions of 'sublimis' tendons in the pollex of lower animals (see Table 2). Superficial flexor tendons and an associated ring are well developed in the pollex of *Mephitis mephitis* (Pl. 2, figs. 14, 15), but in this case, as in the minimus of *Mephitis* and *Erinaceus europaeus*, and the index of *Meles meles*, only the palmaris longus, through easily defined thickenings in the palmar aponeurosis, supplies the contractile element to the tendon ring. In the pollex of *Mephitis* the perforatus tendons are inserted into the palmar ligament of the distal joint, the tendon on the ulnar side being slightly larger than that on the radial aspect.

A single, very weakly developed polliceal 'perforatus' tendon is sometimes found to be blended with the ulnar part of the fibrous flexor sheath in *Felis domestica.* A tendon ring, present in the other digits of this animal, has apparently fused with the fibrous flexor sheath in the pollex, and a slip from palmaris longus passes to this sheath.

Thus perforatus tendons, confined to a digital extent, and attached proximally only to the palmaris longus through a tendon ring, are sometimes present in the digits of quadrupeds, and are occasionally found in the pollex. This probably explains the observation of Parsons (1898) on the mammalian flexor digitorum sublimis—'After that to the pollex, the minimus tendon is the one most frequently wanting, but on several occasions when this has happened, I have found, on splitting up the theca, the perforated loop of the tendon surrounding the flexor perforans, and having its usual insertion, although there was no tendon in the hand and forearm to this digit.'

Particular developments of the pre-axial digit took place amongst the early primates, but specialized locomotion by brachiation stayed this advance amongst the larger members of this order.

Three chimpanzees (*Anthropithecus troglodytes*) have been examined. The flexor pollicis longus tendon is rudimentary in all, taking origin either from the flexor retinaculum or from the flexor profundus tendon to the index. In one specimen, dissected shortly after death, two accessory polliceal tendons were found blending with the metacarpo-phalangeal joint capsule proximally and proceeding to distal insertions deep to, and on either side of, the functionless long flexor tendon (Pl. 3, fig. 16). The tendon on the radial side is relatively poorly developed and is partly fused with the fibrous flexor sheath; that on the ulnar side is quite discrete and lies within the sheath. Immediately prior to their distal insertion fibres decussate between these two tendons, dorsal to the flexor pollicis longus. In the other two specimens only the ulnar-sided accessory tendon can be clearly distinguished, but their state of preservation is not as good as that of the first animal. No other record has been found of these rudiments.

In these primates the abductor et flexor pollicis brevis and the adductor pollicis obliquus send small *extra-vaginal* tendons to the ungual phalanx, not to be confused with the *intra-vaginal* tendons described here. An extension from the abductor was reported by Hepburn (1892) and Fick (1925), from the flexor pollicis brevis by Vrolik (1841) and Humphry (1867), and from the adductor by Gratiolet & Alix (1866) and Sutton (1883).

Accessory intra-vaginal tendons, inserted deep to, and on either side of, the vestigial long flexor tendon were found in the thumbs of a gorilla (*Gorilla gorilla*) and an orang (*Simia satyrus*). In the former the adductor pollicis has gained a partial attachment to the ulnar-sided tendon. In the orang the flexor pollicis longus tendon is embedded proximally in the flexor pollicis brevis muscle, and the adductor pollicis and flexor pollicis brevis are attached to the accessory intra-vaginal tendons.

An intra-vaginal extension to the terminal phalanx in the orang was stated to come from the adductor by Broca (1869), from the inner head of the flexor pollicis brevis by Langer (1879) and Bischoff (1880), from the outer head of the latter muscle by Fick (1895) and from the adductor obliquus by Primrose (1900). These distal prolongations have often been regarded as representing the flexor pollicis longus tendon, e.g. by Langer (1879), but Straus (1942) has shown that the slip which Langer described can co-exist with other rudiments of the long flexor tendon.

Those tendons which are accessory to the flexor pollicis longus and lie within the fibrous flexor sheath in these primates are probably remnants of polliceal perforatus tendons. These accessory tendons do not pass into the antebrachium, but a similarly restricted proximal extent is commonly found in the perforatus tendons of quadrupeds and it is also a characteristic of homologous human rudiments, to be described below. The *ulnar*-sided perforatus tendon in the thumb is the largest and most discrete in the human foetus, in quadrupeds and in the anthropoid apes examined. The latter are not truly 'perforatus' tendons in that they do not extend on to the volar aspect of the long flexor tendon. This feature is, however, a characteristic of the polliceal perforatus tendons of *Mephitis*, and also of the human foetus; moreover the flexor perforatus tendons of the little toe occasionally fail to extend on to the ventral surface of the long flexor tendon.

The position of insertion of these accessory intra-vaginal tendons and their distal relationship to the flexor pollicis longus is *paralleled in man by the lateral tendon fasciculi.*

(4) *The polliceal 'perforatus' tendons in man*

A. *The human foetus*

Frazer (1907) described the existence of a band of cells in the 3 months' foetus arising from the flexor digitorum sublimis above the wrist which came into contact with the *ulnar* side of the flexor pollicis tendon below the wrist, inclined on to the dorsal aspect of this tendon in the thumb and was attached to *both sides* of the terminal phalanx adjacent to the insertion of the long flexor tendon. He maintained that it could not be traced microscopically in the last few months of foetal life, but stated that '...after birth the band can sometimes be distinguished in my opinion by naked eye'. Gräfenberg (1905) suggested that '...in early ontogenesis...it is not impossible that from the superficial common finger flexor, elements are radiating in the direction of the tendon to the first digit'. The only other reports of a human superficial polliceal flexor concern the flexor accessorius ad pollicem of Ganzer (e.g. Fürst, 1900); but this connexion between the superficial and deep flexor layers is the remnant of the condylo-radial head of the primitive *deep* flexor (Windle, 1889), and is not directly relevant to the problem.

In this investigation, serial sections of the manus and lower forearm of four foetuses (C.R. lengths: 35, 57, 70 and 80 mm.) have been examined. Definitive tendons are not present in the 35 mm. foetus. In all the other specimens there is an accessory tendon on the *ulnar* side of the flexor pollicis tendon. It is particularly marked in the 80 mm. foetus (Pl. 3, fig. 17). This arrangement differs from the account given by Frazer in three ways:

(1) The small tendon fuses with the palmar portion of the capsule of the distal joint and one cannot distinguish between the constituent cells of this tendon and the joint capsule distal to this attachment.

(2) The accessory tendon does not exist proximal to the head of the first metacarpal bone.

(3) At this level a weakly developed ring of cells extends from the ulnar-sided tendon and encircles the flexor pollicis longus tendon.

B. *The human adult*

In an examination of forty-two adult thumbs, remnants of a flexor perforatus were found in twelve, i.e. bilaterally in six cadavers. This gives the rather surprising proportion of 28·6 % of the total.

Pl. 3, fig. 18, is a photograph of the right hand of one of these specimens. There is no difficulty in identifying the perforatus tendons, not any danger of confusing them with the general fascia in this region. They are intra-vaginal, and are discrete and glistening except at their most proximal and distal extremities. Proximally they fuse with the parietal synovial membrane on either side of the long tendon opposite the metacarpo-phalangeal joint: sometimes they are also loosely attached to the flexor pollicis longus tendon. They unite more distally, deep to the latter, and are inserted as a single slender tendon into the *ulnar* part of the volar aspect of the palmar ligament of the distal joint.

These rudimentary perforatus tendons cannot be traced proximal to the head of the first metacarpal bone and they have no connexion with the flexor digitorum sublimis. This restricted extent is in harmony with observations on the human foetus and on lower mammals.

It is possible that Frazer chanced upon a very transient stage in the development of these perforatus tendons, not present in the foetal specimens investigated here. It is interesting to note that he described these tendons as having distal insertions which we now recognize as identical in position with the accessory polliceal tendons in apes and with the lateral fasciculi of the flexor pollicis longus tendon in the human adult.

SUMMARY AND CONCLUSIONS

1. Lateral fasciculi arise from the deep surface of the flexor pollicis longus tendon and are inserted into the volar surface of the base of the terminal phalanx on each side of the main portion of the long flexor tendon. The discrete nature of the fasciculi is demonstrated by histological section of the tendon, and by a study of their osseous, vascular and synovial relationships.

2. There are strong reasons for believing that the terminal phalanx of the pollex represents a fusion of two phalanges.

3. Reduction of the middle phalanx in little toes showing phalangeal fusion is associated with reduction of the associated flexor perforatus tendon. Under these conditions the flexor perforans tendon commonly provides a tendon or tendons to the base of the conjoined phalanx.

4. Digital perforatus tendons in quadrupeds are often attached to a mobile tendon ring at the level of the metacarpo-phalangeal joint. The tendons which are inserted proximally into this ring come from diverse sources. Sometimes, particularly in marginal digits, the perforatus tendons have no attachment to the flexor digitorum sublimis, the palmaris longus providing the only contractile element to their associated tendon ring. Polliceal perforatus tendons of this nature are occasionally present and extend distally to the terminal joint capsule. The tendon on the ulnar side is commonly larger than that on the radial side.

5. In human foetuses of 3 to 3½ months a polliceal perforatus tendon lies on the ulnar side of the flexor pollicis longus tendon. It extends from the level of the metacarpo-phalangeal joint, where there are traces of a tendon ring, to the capsule of the distal joint. This structure has also been identified in 28·6 % of adult specimens.

6. Vestigial polliceal perforatus tendons are present in some anthropoid apes. They extend from the level of the head of the first metacarpal bone; the ulnar-sided tendon is the larger, more discrete, and more constant than that on the radial side. They decussate with one another dorsal to the flexor pollicis tendon and are inserted into the terminal phalanx in positions occupied in man by lateral tendon fasciculi.

The function of tendon rings clarifies the unusual disposition of human polliceal perforatus rudiments. In the absence of any direct evidence of a palmar tendon from the flexor digitorum sublimis to the pollex, it is suggested that the palmaris longus was probably attached to the tendon ring of this digit in man's forerunners: this is the arrangement of similarly isolated perforatus tendons in quadrupeds. A strong ligamentous band from the palmar aponeurosis in man to the level of the polliceal metacarpo-phalangeal joint would necessarily become attenuated by the development of an independent mobility of this digit. Thus the digital portions of the perforatus tendons would become truly isolated, functionless and eventually vestigial. The proximal parts of the perforatus tendons in the human thumb have undergone this regression. The flexor pollicis longus having utilized the insertions of these tendons to extend its own lower attachments, the distal portions of the polliceal perforatus tendons have become incorporated into the structure of the long flexor tendon as lateral fasciculi.

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REFERENCES

Albinus, B. S. (1749). *Tables of the Skeleton and Muscles of the Human Body*, 20th table. (Translation.) London: Knapton.

Bardeleben, K. von (1894). On the bones and muscles of the mammalian hand and foot. *Proc. zool. Soc. Lond.* pp. 345–376.

Bichat, De X. (1819). *Traité d'Anatomie descriptive*, **2**, 238. Paris: Brosson, Gabon.

Bischoff, L. W. (1880). Beiträge zur Anatomie des Gorilla. *Abh. bayer Akad. Wiss.* **13**, Abt. 3, 1–48.

Boyer, M. le Baron (1815). *Traité Complet d'Anatomie*, 4th ed., **2**, 299. Paris: Migneret.

Bradley, O. C. (1912). *A Guide to the Dissection of the Dog*, p. 135. London: Longmans, Green and Co.

Broca, P. (1869). L'Ordrê des Primates. *Bull. Soc. Anthrop. Paris*, 2nd series, **4**, 228–401.

Chemin (1896). La synoviale tendineuse. *Bibliogr. anat.* **4**, 132–144.

Cloquet, J. (1822). *Manuel d'Anatomie du Corps Humaine*, p. 195. Paris: Crochard.

Dobson, G. E. (1882). *A monograph on the Insectivora*, Part 1, pp. 50, 51. London: John van Voorst.

Douglas, J. (1763). *Myographiae Comparatae*, p. 130. London and Edinburgh: Donaldson.

Fick, R. (1895). Vergleichend anatomische Studien an einem erwachsenen Orang-Utan. *Arch. Anat. Physiol., Lpz.*, pp. 1–100.

Fick, R. (1925). Beobachtungen an den Muskeln einiger Schimpansen. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **76**, 117–141.

Fourcroy (1785). Mémoire pour servir à l'histoire anatomique des tendons, dans lequel on s'occupe spécialement de leurs capsules muqueuses. *Hist. de l'acad. des Sciences*, 98, 392. Paris.

Frazer, J. E. (1907). Anmerkung über den Bau und die Entwicklung der Sehne des Flexor longus pollicis. *Arch. Anat. Physiol., Lpz.*, pp. 225–226.

Fürst, C. M. (1900). Ein Fall von verkürzten und zweigliedrigen Fingern, begleitet von Brustmuskeldefecten und anderen Missbildungen. *Z. Morph. Anthr.* **2**, 56–76.

Galen (a.d. 131–200). Opera Omnia (1609). *De ossibus.* Tom. 11, Cap. xix, Class 1 and 2.

Geelvink, P. (1913). Ueber hyperphalangie. *Arch. Psychiat. Nervenkr.* **52**, 1015–1029.

Gräfenberg, E. (1905). Die Entwicklung der Knochen, Muskeln und Nerven der Hand und der für die Bewegungen der Hand bestimmten Muskeln des Unterarms. *Arb. anat. Inst., Wiesbaden*, Heft 90, Bd. 30, Abt. I, 1–150.

Grant, J. B. (1947). *An Atlas of Anatomy.* 2nd ed. London: Baillière, Tindall and Cox.

Gratiolet, L. P. & Alix, P. H. E. (1866). Recherches sur l'anatomie du Troglodytes Aubryi. *Nouv. Arch. Mus. Hist. nat. Paris*, **2**, 1–264.

Haines, R. W. (1950). The flexor muscles of the forearm and hand in lizards and mammals. *J. Anat., Lond.*, **84**, 13–29.

Hepburn, D. (1892). The comparative anatomy of the muscles and nerves of the superior and inferior extremities of the anthropoid apes. *J. Anat., Lond.*, **26**, 149–186.

Howell, A. B. (1932). The saltatory rodent Dipodomys. *Proc. Amer. Acad. Arts Sci.* **67**, no. 10, 377–536.

Humphry, G. M. (1867). On some points in the anatomy of the chimpanzee. *J. Anat., Lond.*, **1**, 254–268.

Humphry, G. M. (1869). The myology of the limbs of the unau, the aï, the two-toed anteater and the pangolin. *J. Anat., Lond.*, **4**, 17–78.

Humphry, G. M. (1872). *Observations in Myology*, p. 64. Cambridge and London: Macmillan.

Huxley, T. H. (1871). *A Manual of the Anatomy of Vertebrated Animals*, p. 335. London: Churchill.

Joachimsthal (1898). Ueber Brachydactylie und Hyperphalangie. *Virchow's Arch.* **151**, 429–438.

Joachimsthal (1900). Verdoppelung des linken Zeigefingers und Driegliederung des rechten Daumens. *Berl. klin. Wschr.* **37**, 835–838.

Jones, F. Wood (1949). *The Principles of Anatomy as seen in the Hand*, 2nd ed. reprint, p. 50. London: Baillière, Tindall and Cox.

Jurčić, F. (1906). Ein Fall von Hyperphalangie beider Daumen. *Arch. klin. Chir.* **80**, 562–566.

Krause, W. (1896). Entwickelung der Extremitätenknochen. Bardeleben's *Lehrbuch der Anatomie des Menschen*, Bd. 1, Abt. 3, 77–84. Jena: Fischer.

Kristjansen, A. (1926). Om Hyperphalangia pollicis. *Hospitalstidende*, **69**, 109–119.

Kükenthal, W. (1888). Über die Hand der Cetaceen. *Anat. Anz.* **3**, 638–646.

Langer, C. (1879). Die Musculatur der Extremitäten des Orang als Grundlage einer vergleichend-myologischen Untersuchung. *S.B. Akad. Wiss. Wien*, Abt. 3, 79, 177–222.

Lapidus, W. P. & Guidotti, F. P. (1943). Tri-phalangeal thumb. *Surg. Gynec. Obstet.* **77**, 178–186.

Lucien (1910). Les gaines synoviales carpiennes des fléchisseurs des doigts chez l'homme. *Bibliogr. anat.* **20**, 70–79.

Meckel, J. F. (1825). *Manuel d'Anatomie*, **2**, 176. Paris: Baillière.

Mivart, St G. (1881). *The Cat*, p. 150. London: John Murray.

Parsons, F. G. (1898). The muscles of mammals with special reference to human myology. *J. Anat., Lond.*, **32**, 721–752.

Pfitzner, W. (1890). Die kleine Zehe. *Arch. Anat. Physiol. Lpz.* (*Anat. Abt.*), 12–41.

Pfitzner, W. (1896). Beiträge zur Kenntniss des Menschlichen Extremitätenskelets. *Morphol. Arb. Jena*, **6**, 245–527.

Pfitzner, W. (1898). Ueber Brachyphalangie und Verwandtes. *Vehr. anat. Ges. Jena*, **12**, 19–23.

Pfitzner, W. (1900). Beiträge zur Kenntniss des menschlichen Extremitätenskelets. *Z. Morph. Anthr.* **2**, 365–678.

Poirier, P. & Charpy, A. (1901). *Traité d'Anatomie Humaine*, 2nd ed., vol. **2**, Pt. 1, 118–120, 183. Paris: Masson et Cie.

Primrose, A. (1900). The anatomy of the orang outang. *Univ. Toronto Stud. anat.* no. 1.

Retterer, E. (1896). Sur le développement morphologique et histologique des bourses muqueuses et des cavités péri-tendineuses. *J. Anat., Paris*, **32**, 256–300.

Retterer, E. (1918*a*). Des gaines digitales péritendineuses de l'homme. *C.R. Soc. Biol., Paris*, **81**, 464–467.

Retterer, E. (1918*b*). Connexions des tendons du perforé et du perforant du chien, et leur structure fibro-cartilagineuse. *C.R. Soc. Biol., Paris*, **81**, 521–524.

Rieder, H. (1900). Eine Familie mit dreigliedrigen Daumen. *Z. Morph. Anthr.* **2**, 177–197.

Salzer, H. (1897). Zwei Fälle von dreigliedrigem Daumen. *Anat. Anz.* **14**, 124–131.

Shepherd, F. J. (1883). Short notes on the myology of the American Black Bear (*Ursus americanus*). *J. Anat., Lond.*, **18**, 103–117.

Sisson, S. (1910). *A Text-Book of Veterinary Anatomy*, p. 323. Philadelphia and London: Saunders.

Sobotta, J. (1948). *Atlas der Deskriptiven Anatomie des Menschen*. Berlin-München: Urban and Schwarzenberg.

Spalteholz, W. (1901). *Hand Atlas of Human Anatomy*. Leipzig: Hirzel.

Straus, W. L. (1942). Rudimentary digits in Primates. *Quart. Rev. Biol.* **17**, no. 3, 228–243.

Sutton, J. B. (1883). On some points in the anatomy of the Chimpanzee (*Anthropithecus troglodytes*). *J. Anat., Lond.*, **18**, 66–85.

Toldt, C. (1944). *An Atlas of Human Anatomy*. New York: Macmillan.

Vesalius, A. (1555). *De Humani Corporis Fabrica*. Lib. 1. De pedis ossibus. De ossibus digitorum manus. Basle: Oporinum.

Vrolik, W. (1841). *Recherches d'anatomie comparée sur le chimpansé*, p. 20. Amsterdam: Müller.

Wakeley, C. P. G. (1924). Bilateral epiphysis at the basal end of the second metacarpal. *J. Anat., Lond.*, **58**, 340–345.

Welcker, H. (1884). *Die Morphologische Bedeutung des ersten Daumengliedes*. Separatabdruck aus dem Preisvertheilungsprogramm der Universität, Halle.

Whittaker, C. R. (1907). The arrangement of the synovial membrane in the palmar digital sheaths. *J. Anat., Lond.*, **41**, 155–158.

Wilkinson, J. L. (1951). The anatomy of an oblique proximal septum of the pulp space. *Brit. J. Surg.* **38**, 454–459.

Windle, B. C. A. (1889). The flexors of the digits of the hand. *J. Anat., Lond.*, **24**, 72–84.

Windle, B. C. A. (1891). The occurrence of an additional phalanx in the human pollex. *J. Anat., Lond.*, **26**, 100–116.

Windle, B. C. A. & Parsons, F. G. (1897). On the myology of terrestrial carnivora. *Proc. zool. Soc. Lond.* pp. 370–409.

Winslow, J. B. (1733). *An Anatomical Exposition of the Structure of the Human Body*. English translation by G. Douglas. Vol. I, Sect. 3, p. 43. London: G. Bettesworth and C. Hitch.

EXPLANATION OF PLATES

Plate 1

Fig. 1. The distal insertions of a flexor digitorum profundus tendon. × 1·6.

Fig. 2. The distal insertions of a flexor pollicis longus tendon. *f*, lateral fasciculi. × 1·3.

Fig. 3. The distal insertions of a flexor pollicis longus tendon. *f*, lateral fasciculi. × 1·7.

Fig. 4. Transverse $100\,\mu$ sections of the distal 1·5 cm. of a flexor pollicis longus tendon. *f*, lateral fasciculi. Approximate levels of sections: (*a*) head of subterminal phalanx; (*b*) terminal articulation; (*c*) base of distal phalanx. Haematoxylin and eosin. × 5·6.

Fig. 5. Terminal polliceal phalanges. The roughening associated with the insertion of the long flexor tendon has been marked with indian ink in (*a*) and (*b*). The bristles in (*b*) and the arrows in (*c*) indicate the positions of vascular foramina. × 1·1.

Fig. 6. Lateral radiograph of a thumb, flexed at the terminal joint. × 1·1.

Fig. 7. Terminal phalanges of fingers. The roughening for the insertion of the flexor digitorum profundus has been marked with indian ink in (*b*). Note the proximally projecting tips of the tuberositas unguicularis. In (*a*) there is also a spike (*s*) which represents the proximal attachment of the ligament which extends between the base of the phalanx and the tuberositas unguicularis. × 1·2.

Plate 2

Fig. 8. Radiographs of the distal portion of the lumen of the injected synovial sheath of a flexor pollicis longus tendon. *d*, lateral diverticula of sheath; *m*, mid-line cleft. × 1·3.

Fig. 9. Radiographs of the injected synovial sheath of the flexor tendons of an index finger. *d*, small diverticula where the flexor digitorum sublimis tendons leave the sheath.

Fig. 10. A right little toe with fused phalanges. A groove (*g*) marks the site of fusion of the middle and terminal phalanges. The long flexor tendon provides a fasciculus (*f*) to the base of the conjoined phalanx. × 1·3.

Fig. 11. Distal fusion of flexor tendons in a little toe: phalangeal fusion. *pu*, perforatus tendons. *pa*, perforans tendon. × 1·2.

Fig. 12. Right little toe. The long flexor tendon supplies two fasciculi (*f*) to the middle phalanx. No phalangeal fusion.

Fig. 13. A comparison between the insertion of a flexor pollicis longus tendon (*a*, × 1·5) and the flexor tendons of a little toe (*b*, × 2). Note the similarity in disposition of the lateral tendon fasciculi (*f*) in the thumb and the perforatus tendons (*pu*) in the toe.

Fig. 14. A magnified view of the volar aspect of the left pollex and index of *Mephitis mephitis*. The deep flexor tendons have been cut and retracted distally. *Ppu*, polliceal perforatus tendons; *Ipu*, perforatus tendons of the index; *C*, volar surface of the distal joint capsule. *Pl*, palmaris longus tendons; *Fds*, flexor digitorum sublimis tendon; *Pr*, long flexor tendons of the terminal phalanges. × 5·4.

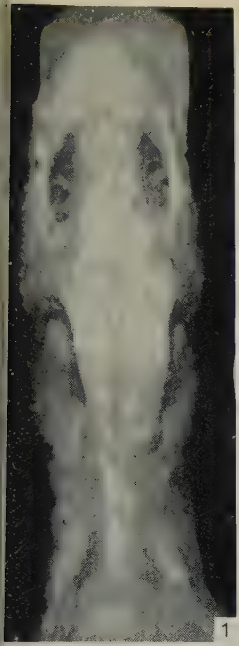
Fig. 15. Diagrams of the tendon rings in the index and pollex of *Mephitis*. Abbreviations as in fig. 14.

PLATE 3

Fig. 16. A dissection of the right pollex and thenar eminence of *Anthropithecus trogolodytes*. *Fpl*, flexor pollicis longus tendon. *Acc*, large ulnar and small radial-sided accessory tendons to the terminal phalanx. *Eaf*, distal extension of the abductor et flexor pollicis brevis. *Eo*, distal extension of the adductor obliquus. × 0·8.

Fig. 17. Transverse 10 μ sections of the right flexor pollicis longus tendon of an 80 mm. human foetus. *Acc*, ulnar-sided accessory tendon. *R*, ring of cells. *P*, proximal phalanx. *M*, first metacarpal. × 46.

Fig. 18. A rudimentary perforatus tendon (*Pu*) in the right pollex of an adult male cadaver. *Sy*, parietal layer of the synovial flexor sheath.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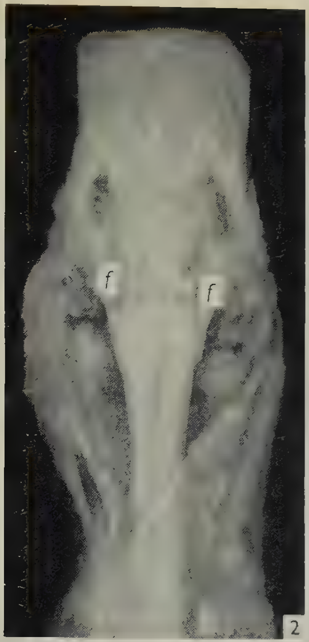
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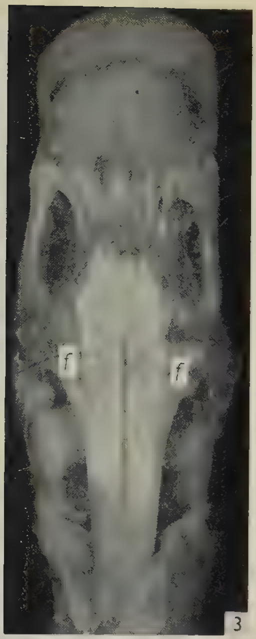
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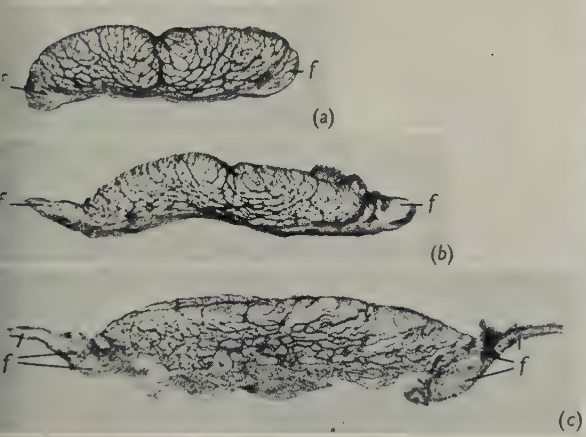

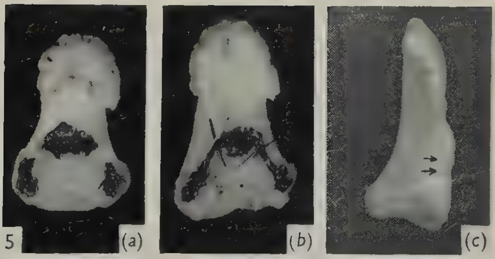
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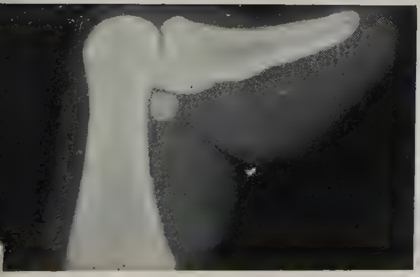

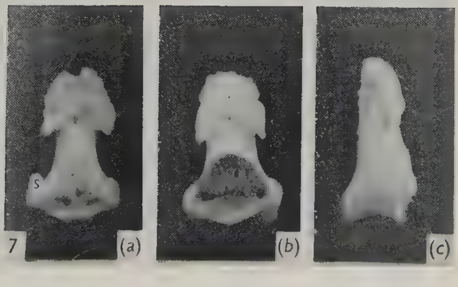
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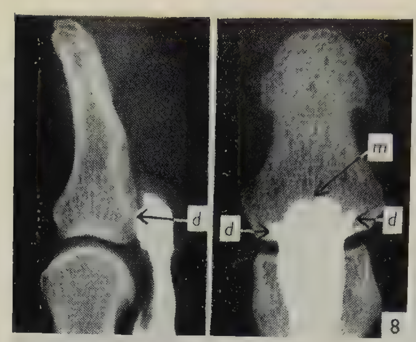
ILKINSON—THE INSERTIONS OF THE FLEXORES POLLICIS LONGUS ET DIGITORUM PROFUNDUS



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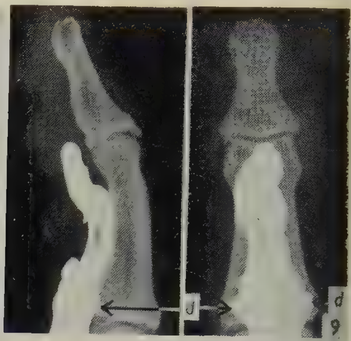

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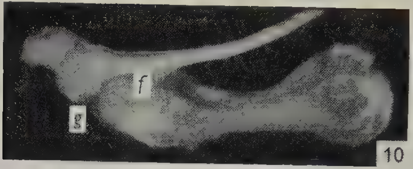

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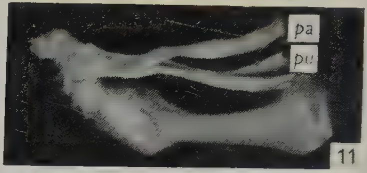

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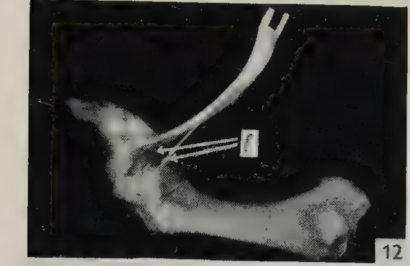

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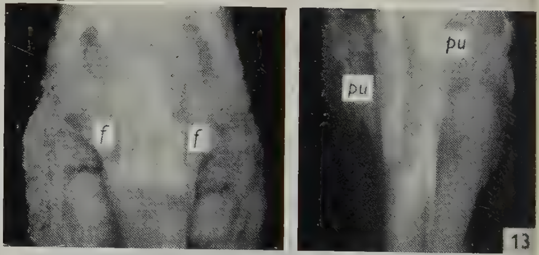

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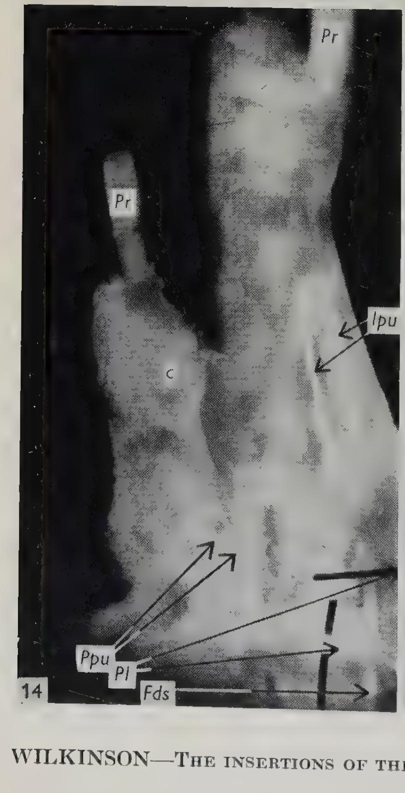

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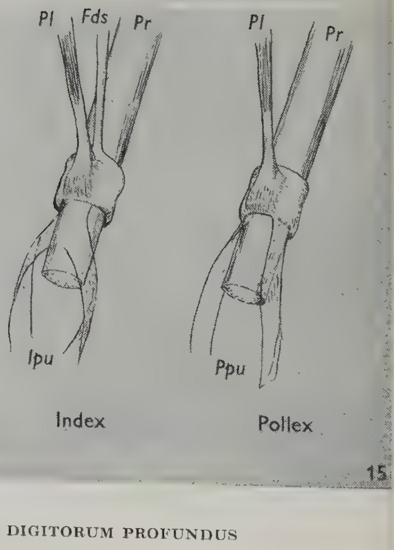

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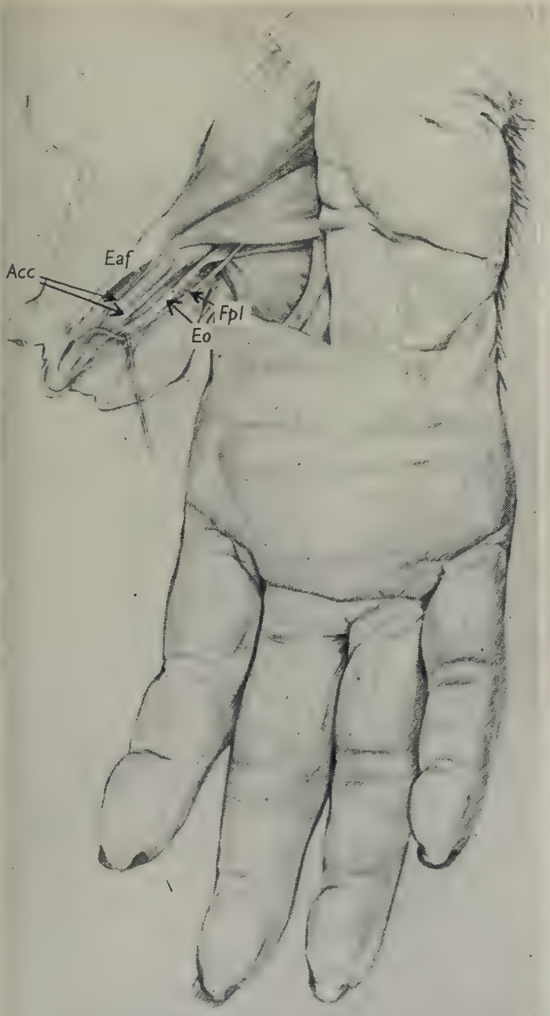
WILKINSON—THE INSERTIONS OF THE FLEXORES POLLICIS LONGUS ET DIGITORUM PROFUNDUS



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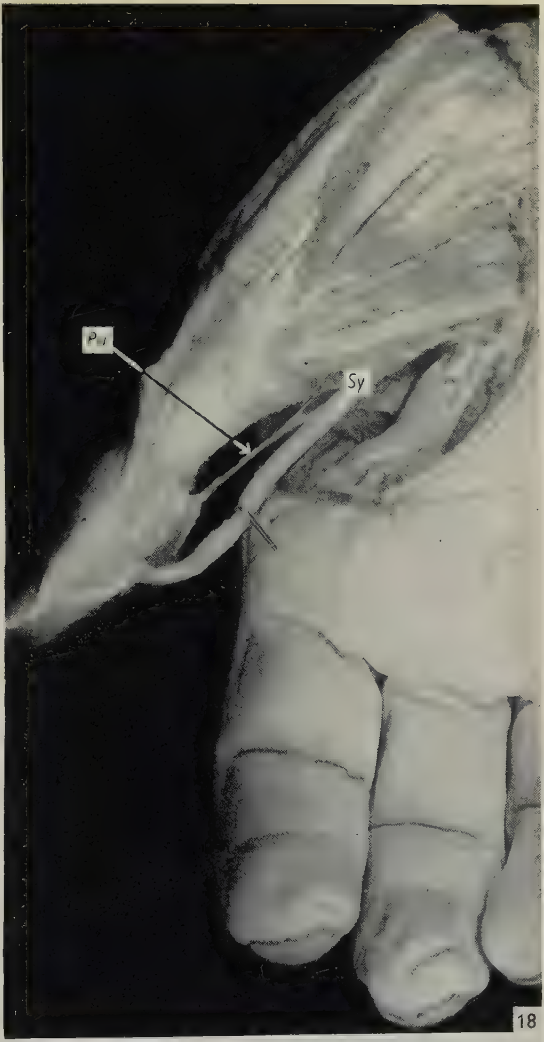

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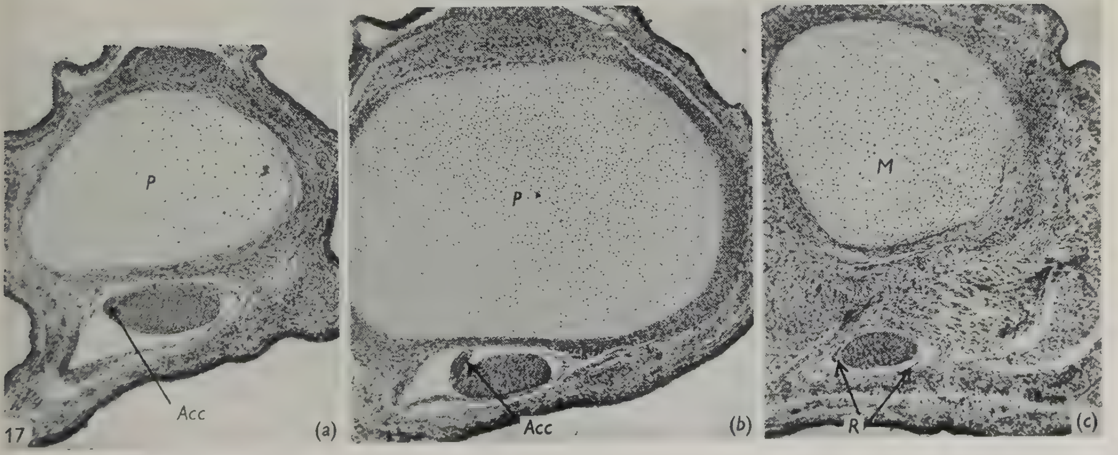

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WILKINSON—THE INSERTIONS OF THE FLEXORES POLLICIS LONGUS ET DIGITORUM PROFUNDUS

REVIEWS

Growth of Children. Sixty-six Boys and Sixty Girls each Measured at Three Days, and at One, Two, Three, Four and Five Years of Age. By ALEXANDER LOW. (Pp. 64. 10*s.* 0*d.*) University of Aberdeen. 1952.

It has long been recognized that the growth of animals can only be adequately studied from repeated measurements made on the same animals at successive ages, the so-called longitudinal survey. It is very difficult to make such a survey where human beings are concerned, and although data of this kind have been gathered in America, little similar work has been carried out on the people living in Britain. Students have known for some time that the late Prof. Low had made a number of measurements on young children and the publication of his records has been eagerly awaited. Some of these, edited by members of the Anatomy Department of the University of Aberdeen, are published in the pamphlet under review. This contains 46 pages of Tables of Measurements, 10 pages of the values of certain indices computed from the data, an illustration of a typical record card and is prefaced by a single page describing the methods of measurement. Data concerning boys and girls are on opposite pages and particulars of the ages of the parents, the number of the pregnancy, type of infant-feeding and such like are provided. The measurements were made 3 days after birth, at 1, 2 and 3 years old, and, in the majority of cases, at 4 and 5 years of age as well. These intervals are very wide for an animal changing its shape and size as rapidly as a child during the first five years after birth. In a subject where strictness of standard is so important, the single page devoted to the description of the methods used in this inquiry is disappointing.

The most important measurements are weight and height. The weighings only appear to have been accurate to the nearest quarter of a pound. The measurements of height are even more open to question. The Table on p. 28 is headed 'Standing Height of Male Children in mm.'; it is clear that such a measurement is impossible on a child 3 days old. Reference to the page on Methods tells us that 'Height measurements were made up to the age of three years on the measuring board, and subsequently with the child standing erect'. A short description of the measuring board is given, but it is clear that in these Tables we have not a standard method but one kind of length measurement for children under 3 years old and another for the later ages. Furthermore, we are given no information about posture. Were the heels, buttocks and shoulders against a wall? Was the head held in the Frankfurt plane or was the occiput in contact with a wall (only in long-headed subjects can these conditions be satisfied simultaneously)? Similar criticisms apply to the measurements of sitting height. Again, we are told that 'For chest circumference the tape was carried horizontally at the level of the nipples and the reading made with the chest midway between inspiration and expiration.' How is such a midway position to be judged? If such a central reading is desirable it is better to take the mean of the readings at full inspiration and full expiration. What is meant by 'The length of the foot' and by 'Height of tip of middle finger was taken from that point to the sole of the foot'? Many similar questions arise and one feels that the description of the methods should have been greatly amplified if the data were to be useful to workers on human growth.

At the foot of each column of measurements, e.g. of the weights of 3-day-old boys, the mean value and the standard deviation of the observations together with the standard error of the mean are given. In view of the stress laid on the longitudinal nature of the survey this information seems rather pointless and, in fact, may be a trap for the unwary.

The Foreword states that 'Professor Low profoundly disagreed with the current statement that children of today are taller and heavier' and that 'The tables of his data now published provide workers in this field with a reliable basis for further investigation and analysis'. These sentences seem to imply that the data in these Table are suitable for making statements about some population of children, presumably those living in the north-east of Scotland. However, since no indication is given of the sampling methods used, these measurements do not enable any statements to be made about the characteristics of any population.

It is a great pity that these Tables are open to so many grounds of criticism but it is most essential that future workers should realize their shortcomings. Before starting on their measurements, students new to this field would be wise to take advantage of the advice of the Committee on Growth and Form set up by the Medical Research Council.

D. A. SHOLL

Callander's Surgical Anatomy. By BARRY J. ANSON and WALTER G. MADDOCK, 3rd edition. (Pp. xi+1074; 929 illustrations; 7 × 10 in.; 70*s*.). London: W. B. Saunders Co. Ltd. 1952.

Owing to the death of Dr Callander, the new edition of this well-known book has been extensively revised by Prof. Barry J. Anson, Professor of Anatomy, and Prof. Walter G. Maddock, Elcock Professor of Surgery, both of Northwestern University Medical School.

This book, which is divided into ten parts, is comprehensive and is extremely well illustrated by diagrams, drawings and photographs. The aim of the book is to provide the fundamental features of surgical anatomy and not detailed surgical techniques, since these soon become obsolete. In this aim the authors have been extremely successful. It is to be regretted that more details have not been given of the distribution of the bronchial tree and the addition of several bronchograms would have enhanced the value of this section. The authors should also consider whether it would not be advisable to include more radiographs of normal structures in any subsequent edition.

The book is well written and contains very few typographical errors; it can be recommended to the surgeon as well as to the senior student. We have no doubt that this new edition will continue to receive the popularity accorded to its predecessors.

W. J. HAMILTON

BOOK RECEIVED

Vesalius on the Human Brain. Edited and translated by Charles Singer. (Pp. xxvi+151; 47 figs.; 21*s*.) Oxford: The University Press. 1952.

LOCKING AT THE KNEE JOINT

By C. H. BARNETT

Department of Anatomy, St Thomas's Hospital Medical School

INTRODUCTION

Many theories have been advanced in the past to account for the medial rotation of the femur upon the tibia that takes place during the last phase of extension at the knee joint. For example, this 'locking' movement has been ascribed to tautening of the anterior cruciate ligament (Young, 1889), unwinding of the twisted cruciate ligaments (Haines, 1941), an inclined axis of rotation (Meyer, 1853), unequal curvatures of the femoral condyles (Bugnion, 1892), altered weight distribution (Walmsley, 1933), and the inequality of antero-posterior length of the femoral condyles. The last is the most popular view as judged by the accounts in current text-books; the lateral femoral condyle, shorter than the medial, is said to be 'used up' first as the knee extends, and the remainder of the medial condyle comes into apposition with the tibia by sliding backwards. In considering these theories it must be borne in mind that the alleged cause of the medial femoral rotation may be in fact merely its result. If rotation is to occur—whatever the cause—the femoral condyles must inevitably be unequal in length to accommodate the movement, and the attachments of the cruciate ligaments must be so situated as to allow it.

It is questionable whether axial rotation at the knee joint should be dismissed as 'a small movement of no particular functional significance' (Haines, 1941). Many other hinge joints exhibit such rotation, which has been shown to be mechanically advantageous in reducing the frictional wear of articular surfaces (MacConaill, 1946). It is probable that axial rotation of the femur serves to improve the mechanical efficiency of the knee joint, particularly during the phase of extension when, during walking, there is the greatest pressure across the articular surfaces. Moreover, this rotation is of definite clinical importance, for if it is forcibly prevented internal derangement of the knee is likely to result (Smillie, 1951).

The nature of the movements that can occur at any joint depends on the form of the articular surfaces, the restraining influence of ligaments and the control exerted by the muscles acting on the joint, and the locking movement at the knee is no exception (Goodsir, 1855). While the ligaments and the muscles acting at the knee joint have often been discussed, the part played by the articular surfaces in producing axial rotation has received less attention. The present investigation is therefore concerned mainly with this aspect of the problem.

MATERIAL AND METHODS

Three ciné-radiographic films have been made of normal subjects showing axial rotation of the femur as the knee is extended. In one the subject was rising to his feet from a stooping posture; in the other two, the thigh was supported in a horizontal position and the knee was slowly extended, care being taken to keep the long axis of the foot in a vertical plane so that any rotation at the knee would be of the femur upon the tibia and not vice versa.

Twenty knees from dissecting-room subjects have been examined; twelve which showed no pathological changes were selected for detailed measurement. In addition eleven freshly amputated normal knees were used to study the movements of the menisci during flexion and extension.

The curvatures of the articular surfaces were measured either by means of a planimeter or by sawing them through and tracing around the cut edges. It was found that a length of wire moulded to fit the profile of each femoral condyle was useful in assessing the congruity between the femoral condyles and the underlying articular surfaces.

OBSERVATIONS AND DISCUSSION

Cine-radiography clearly shows that while medial rotation of the femur can be detected at least 30° short of full extension, it is maximal during the last 10°. It is clear that the factors bringing about axial rotation must begin to take effect before extension is complete.

Extension of the knee takes place between the femur above and the menisci and tibial condyles below. Rotation, on the other hand, involves movement of the femur and the menisci upon the fixed tibia. It is convenient to consider these movements separately even though, during locking at the knee, they are occurring simultaneously.

The profiles of the medial and lateral femoral condyles are spiral in form, and extension of either condyle must cease as soon as the upper and lower articular surfaces become congruous, in the ‘close-packed’ position (Walmsley, 1928; MacConaill, 1932). In the normal knee, however, close-packing of the lateral condylar surfaces always precedes that of the medial surfaces. There are two reasons for this: firstly, the profile of the lateral femoral condyle flattens, from back to front, more rapidly than that of the medial condyle (Bugnion, 1892); secondly, the lateral receiving surface, i.e. the lateral tibial condyle with the meniscus *in situ*, is more concave than the medial. These differences are clearly seen if sections of the normal knee are cut in the sagittal plane with the menisci in place (Fig. 1).

As the knee is straightened the lateral femoral condyle thus becomes prematurely close-packed and its further extension is necessarily brought to a halt. Medial rotation around a vertical axis is, however, still possible (Walmsley, 1933). The exact location of this vertical axis is disputed and probably varies with the degree of flexion at the knee (Brantigan & Voshell, 1941; Abbott, Saunders, Bost & Anderson, 1944). In the phase at which locking occurs, it appears to pass through the head of the femur above and a point between the tibial attachments of the two horns of the lateral meniscus below. The medial rotation of the femur is brought about by the contracting quadriceps muscle which pulls the upper end of the femoral shaft forward relative to the fixed lateral femoral condyle, thus rotating the bone medially about the vertical axis (Fig. 2).

While the medial femoral condyle glides backwards, the lateral femoral condyle rotates medially about this vertical axis. The lateral meniscus rotates likewise (Weber & Weber, 1843), pivoting about the closely set attachments on the tibia of its anterior and posterior horns. This rotation causes the anterior horn to glide down a cambered surface on the front of the lateral tibial condyle (Fig. 3) and thus the concavity of this part of the receiving surface is reduced. As a result the lateral condylar surfaces are no longer close-packed and further extension is again possible.

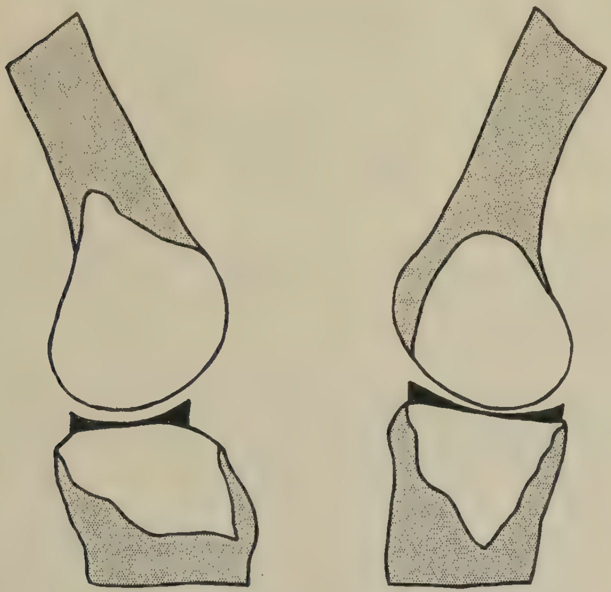

Fig. 1.

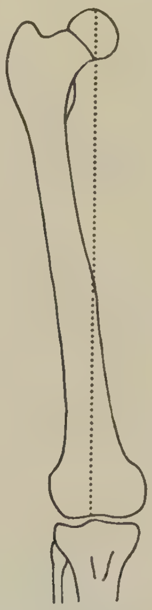

Fig. 2.

Fig. 1. Sagittal sections through the lateral and medial condyles of a normal knee. The stippled portions are excluded from the section. (Drawn from photographs.)

Fig. 2. The vertical axis around which medial rotation of the femur occurs during locking at the knee.

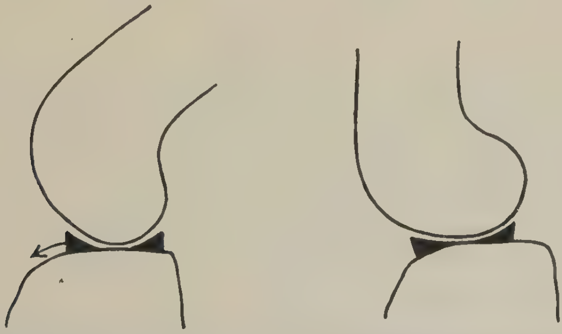

Fig. 3. Diagram illustrating the movement of the lateral meniscus as the knee is extended.

It is obvious that in the normal knee there is no alternation of extension and rotation. The femur is extended steadily and the lateral condylar surfaces are prevented from becoming congruous by the simultaneous recession of the anterior horn of the lateral meniscus. The sequence of events can be visualized more clearly, however, if they are considered as a cycle of alternating extension and rotation as in Table 1.

Table 1

Movement of femur	Effect		Result
	Lateral condyles	Medial condyles	
(1) Extension	Close-packed	Not congruous	Further extension checked
(2) Medial rotation	No longer congruous	Not congruous	Extension again allowed
(3) Further extension	Again close-packed	Not congruous	Extension again checked
(4) Movements (2) and (3) repeated	Close-packed	Eventually close-packed	Full extension with locking

It can be seen that eventually both the lateral and medial condylar surfaces are close-packed in full extension, producing maximal stability. MacConaill (1932) has demonstrated this fact experimentally by observing the interior of normal knee-joints through holes bored in the bones.

Minor incongruities found between the femoral and tibial articular surfaces can be explained in terms of compression of articular cartilage (Hirsch, 1944), peripheral distraction of the menisci (Fairbank, 1948) and release of tension in the ligaments of Humphrey and Wrisberg during extension of the knee (Holtby, 1915).

UNLOCKING OF THE KNEE JOINT

Cine-radiography indicates that lateral rotation of the femur commences as soon as flexion is attempted, and is completed when the knee has undergone only a few degrees of flexion. This rapid movement is presumably brought about by contraction of the popliteus muscle. Preliminary observations, based upon electromyographic recordings from needle electrodes inserted directly into the muscle during weight-bearing, show that activity can be recorded only at the commencement of flexion. The attachment of part of the popliteus tendon to the back of the lateral meniscus (Last, 1948) presumably causes the marked recession of the posterior horn of the lateral meniscus that occurs as flexion of the knee commences (Brantigan & Voshell, 1941).

SUMMARY

Locking at the knee joint—the medial rotation of the femur that occurs as the normal knee is extended—is shown by means of ciné-radiography to commence at least 30° short of full extension, although maximal during the last 10°. In contrast, lateral rotation of the femur as the knee is unlocked is confined to the first few degrees of flexion. This axial rotation probably improves the mechanical efficiency of the joint by reducing the frictional wear of the articular surfaces, especially during walking.

The conformation of the articular surfaces helps to produce this rotation; early close-packing of the lateral condyle as the knee is straightened restricts further extension of the femur until medial rotation, brought about by the contraction of the quadriceps muscle, has occurred.

I am indebted to Prof. D. V. Davies and Prof. M. A. MacConaill for criticism and advice, and to Dr Russell J. Reynolds for his kindness in making the ciné-radiographic films.

REFERENCES

Abbott, L. C., Saunders, J. B. de C. M., Bost, F. C. & Anderson, C. E. (1944). Injuries to the ligaments of the knee joint. *J. Bone Jt Surg.* **26**, 503–521.

Brantigan, O. C. & Voshell, A. F. (1941). The mechanics of the ligaments and menisci of the knee joint. *J. Bone Jt Surg.* **23**, 44–66.

Bugnion, E. (1892). Le mécanisme du genou. Recueil Inaug. de l'Université de Lausanne. Quoted by Poirier, P. & Charpy, A. (1911). *Traité d'Anatomie Humaine*, **1**, 3rd ed. Paris: Masson et Cie.

Fairbank, T. J. (1948). Knee joint changes after meniscectomy. *J. Bone Jt Surg.* 30B, 664–670.

Goodsir, J. (1855). On the horizontal curvature of the internal femoral condyle; on the movements and relations of the patella, semilunar cartilages, and synovial pads of the human knee joint. Contained in *Anatomical Memoirs of the late John Goodsir*, 1868. Edinburgh: Adam and Charles Black.

Haines, R. W. (1941). A note on the actions of the cruciate ligaments of the knee joint. *J. Anat., Lond.*, **75**, 373–375.

Hirsch, C. (1944). A contribution to the pathogenesis of chondromalacia of the patella. *Acta chir. scand.* **90** (Suppl. 83), 1–106.

Holtby, J. R. D. (1915). A note on the posterior cruciate ligament of the knee-joint. *J. Anat., Lond.*, **49**, 386–388.

Last, R. J. (1948). Some anatomical details of the knee joint. *J. Bone Jt Surg.* 30B, 683–688.

MacConaill, M. A. (1932). The function of intra-articular fibrocartilages, with special reference to the knee and inferior radio-ulnar joints. *J. Anat., Lond.*, **66**, 210–227.

MacConaill, M. A. (1946). Studies in the mechanics of synovial joints. III. Hinge-joints and the nature of intra-articular displacements. *Irish J. Med. Sci.* (Sept.), pp. 620–626.

Meyer, H. (1853). Die Mechanik der Kniegelenks. *Arch. Anat. Physiol.* pp. 497–547. Ed. J. Müller. Berlin: Verlag von Veit et Comp.

Smillie, I. S. (1951). *Injuries of the Knee Joint*, 2nd ed. Edinburgh: E. and S. Livingstone Ltd.

Walmsley, T. (1928). The articular mechanism of the diarthroses. *J. Bone Jt Surg.* **10**, 40–45.

Walmsley, T. (1933). The vertical axes of the femur. *J. Anat., Lond.*, **67**, 284–300.

Weber, G. & Weber, E. (1843). Mécanique des organes de la locomotion. In *Encyclopédie Anatomique*, **2**, 2nd ed. Ed. by Bischoff, G. T. *et al.* Paris: J. B. Baillière.

Young, R. B. (1889). On the grooves separating the patellar from the menisco-tibial surfaces of the femur, and on locking of the knee-joint in full extension. *Memoirs and Memoranda in Anatomy*, **1**, 147–158. By Cleland, J., Mackay, J. Y. & Young, R. B. London: Williams and Norgate.

THE OLFACTORY TUBERCLE, PREPYRIFORM CORTEX AND PRECOMMISURAL REGION OF THE PORPOISE (*PHOCAENA PHOCAENA*)

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INTRODUCTION

In recent years considerable doubt has been thrown on the attribution of purely or predominantly olfactory functions to parts of the so-called 'rhinencephalon', e.g. by Brodal (1947). In this connexion, attention has naturally been directed from time to time to the 'rhinencephalon' in anosmatic brains such as that of the dolphin, since in the absence of an olfactory bulb and tracts one might expect to find loss, or at least marked retrogression, of those parts of the brain traditionally regarded as being concerned mainly with olfactory functions.

There are many descriptions of the Cetacean brain available in the literature (Kükenthal & Ziehen, 1889; Addison, 1915; Langworthy, 1932; Jelgersma, 1934; Ries & Langworthy, 1937; etc.) but these are largely confined to the macroscopic appearances, and most of them deal with the rhinencephalon only incidentally; where microscopic methods have been used, e.g. Addison (1915), Jelgersma (1934), the Weigert technique alone has been employed, and by itself this has added very little to what could be seen or inferred from macroscopic examination or dissection. The cellular architecture of the rhinencephalon does not appear to have been studied in any detail, and, while it is realized that the examination of Nissl preparations does not give much fundamental information about the functional organization of the nervous system, this technique has been used so extensively in other mammals that for comparative purposes it is clearly of value and importance.

It is the purpose, therefore, of the present work to extend the basis of comparison between an anosmatic mammal (the porpoise, *Phocaena phocaena*) and other mammals, by investigating the cellular pattern, and, within the limits necessarily imposed by 'normal' material, the fibre connexions, of the rhinencephalon. This paper is confined to a description of those parts of the brain which appear to be comparable to the structures on the basal and medial surfaces which in other mammals are directly related to the olfactory bulb and tracts, namely, the prepyriform cortex, olfactory tubercle, and precommissural region or septum. While it is realized that direct connexions from the olfactory bulb to this latter region have not been demonstrated experimentally in any mammal, it is usually considered to be part of the rhinencephalon and, as such, merits description from the present point of view.

MATERIALS AND METHODS

The material used in this investigation consisted of the brains of two adult porpoises (*P. phocaena*). The animals were shot off the Isle of Cumbrae, and shortly after, the skulls and their contents were sawn through (one in a paramedian plane, P_1, and the other in the coronal plane, P_2), and the resulting 'halves', after immersion in 10% formalin for 3 days, were despatched to London. Upon arrival the brains were removed, treated with 10% formalin for another 3 days, photographed, and cut into slices, from which blocks were taken and embedded in celloidin. Two series of sections (at 20μ) were prepared, and the plane of section of the one from which most of the drawings and photographs were taken is indicated in Text-fig. 7; the plane of the other was approximately at right angles to this. Alternate sections were stained with thionin, and by Weil's modification of the Weigert method. In some regions every section was examined, in others, examination of every fifth or tenth was considered sufficient.

MACROSCOPIC APPEARANCES

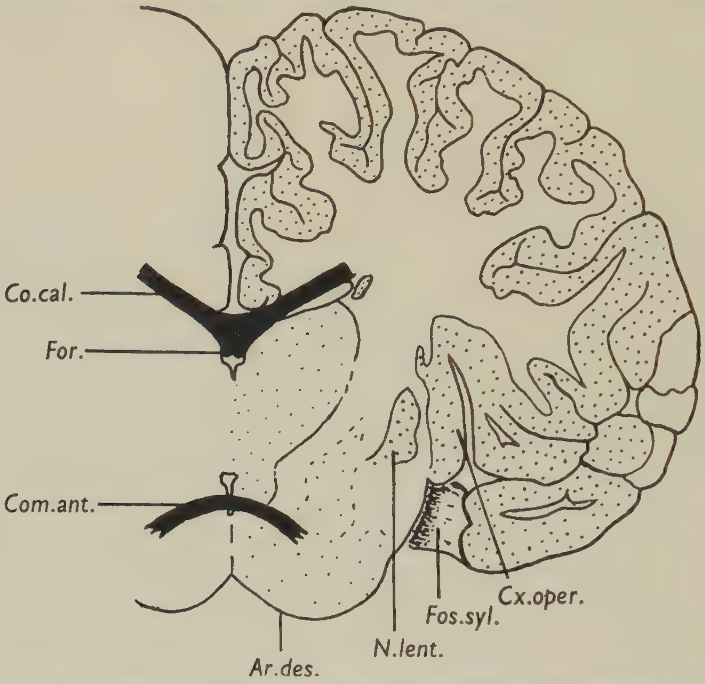
Ventral aspect (Pl. 1, fig. 1)

There is a complete absence of olfactory bulbs and tracts. In front of the optic tract, in the region occupied by the olfactory tubercle in other mammals, is an extensive convex area which extends laterally into the Sylvian fossa (Text-fig. 1). It is easily distinguished from the surrounding cortex by its somewhat paler colour, and the surface is pitted by numerous vascular foramina, the majority of which are concentrated postero-laterally. This will be called the 'area désert', following Broca's use of the term 'désert' to denote an area free from convolutions. Medially, it is continuous around the ventro-medial border of the hemisphere with the precommissural area, and it is limited anteriorly and laterally by a curved sulcus marked *a*, in Pl. 1, fig. 1, the anterior part of which is poorly defined. As this sulcus is traced laterally and posteriorly it becomes more pronounced, and disappears into the depths of the Sylvian fossa, within which it bends back on itself to continue anteriorly for some distance on the dorsal surface of the temporal lobe. Along the line of this sulcus, fibres can be seen to reach the surface and to extend medially for varying distances.

This area is a characteristic feature of many cetacean brains. In the dolphin, it was divided by Addison (1915) into a 'lobule désert of Broca', and a 'substantia perforata anterior'. In the porpoise, such a subdivision does not appear to be justified, at least on the macroscopic appearances. As indicated by Addison (1915), Ries & Langworthy (1937), and others, its prominence is largely due to the size of the underlying caudate nucleus. Within its limits Addison tentatively identified 'the last remnants of the lobus parolfactorius' (olfactory tubercle), as well as a formation which he considered might represent the remains of the olfactory lobe (prepyriform cortex). Jelgersma (1934) stated that it was largely occupied by an extensive mass of degenerated tissue, devoid of nerve cells. These observations were based on the study of Weigert preparations, and were subject to its limitations; neither author was in a position to give a detailed account of the cellular configuration of the region.

Beyond the limiting sulcus of the area désert are two gyri marked *X* and *Y* in Pl. 1, fig. 1. Gyrus *X* runs transversely, anterior to the area, and is indistinctly separated from the cortex of the under surface of the frontal lobe by a shallow sulcus (*b*, Pl. 1, fig. 1). At its lateral end, it bends posteriorly to become continuous with the medial part of the gyrus *Y*. The latter can be followed back into the Sylvian fossa, where it becomes continuous with the cortex of the temporal lobe. In coronal section (Text-fig. 1) gyrus *Y* can be seen to form the ventral edge of an operculated cortical area which macroscopically bears many similarities to the human insula.

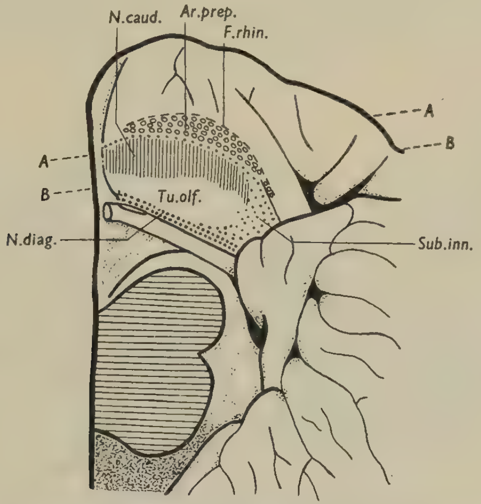
These appearances closely resemble those illustrated by Addison (1915), Jelgersma (1934), and Ries & Langworthy (1937), but none of these authors express any views as to the homologies of the gyri and sulci described. From the macroscopic appearances alone, there might be some justification for regarding the gyri *X* and *Y* as representing the lateral olfactory gyrus or prepyriform lobe of other mammals. In *Balaenoptera sulphurea*, which possesses an olfactory peduncle, Wilson (1933) identified a lateral olfactory gyrus in such a position, but great caution is necessary in accepting homologies between gyri which are based on macroscopic evidence only. This is particularly important when dealing with brains such as those of the Cetacea, which are profoundly modified in general shape compared with other mammals. The difficulty is greater in the porpoise where the landmarks provided by olfactory bulbs and peduncles are absent.



Text-fig. 1. Drawing of an oblique section through the area désert to show its lateral extension into the Sylvian fossa, and the operculated cortex. Natural size.

Medial aspect (Pl. 1, fig. 2)

Ventral to the genu of the corpus callosum is a smooth area which corresponds to the region variously described in other mammals as 'septum', 'precommissural area', 'paracommissural region', etc. Ventrally, it is continuous around the ventro-medial margin with the area désert, and anteriorly appears to be limited by the prolongation of the supra-callosal sulcus towards the frontal pole. Posteriorly, it is limited by the optic chiasma, lamina terminalis (containing the small anterior commissure), and the genu of the corpus callosum. Owing to the absence of a rostrum, it is continuous between the genu and the anterior column of the fornix with the corresponding leaf of the septum pellucidum, and between the two leaves there is a well-marked cavum septi of the 'open' (Thompson, 1932) variety. In macrosmatic mammals, the precommissural area overlies the medial and lateral septal nuclei, the nucleus accumbens, the anterior hippocampal cortex, and various fibre systems, including the precommissural fornix, all of which have been thought to be concerned with olfaction. From the point of view of the present investigation, therefore, the microscopic structure of this region is of importance.



Text-fig. 2. Drawing of the ventral aspect of the brain of $P2$ to show the extent of the prepyriform cortex and the structures occupying the surface of the area désert. The rhinal fissure is represented by the broken line along the antero-lateral edge of the prepyriform cortex; it corresponds with sulcus *b* (Pl. 1, fig. 1). The lines *AA* and *BB* show where the sections illustrated in Text-figs. 3 and 4 cut the ventral surface of the brain.

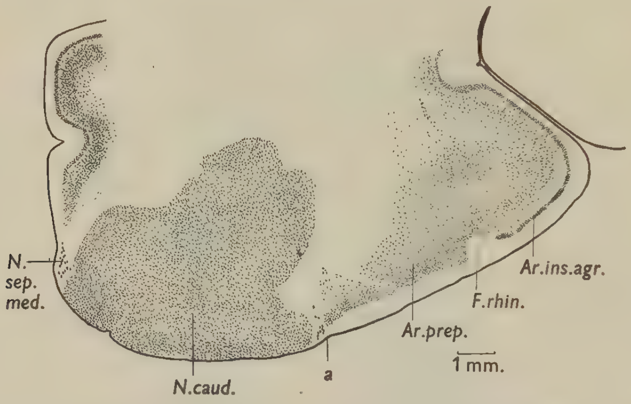
MICROSCOPIC APPEARANCES

The cortex anterior and lateral to the area désert

In front of the area désert a narrow strip of cortex having a characteristic microscopic structure can be defined. Its extent is illustrated in Text-fig. 2 (*Ar. prep.*), where it can be seen to occupy the whole of the gyrus *X*, and to send a narrow extension laterally along the medial edge of the gyrus *Y*. This lateral extension becomes progressively narrower as it is traced posteriorly, but in one brain it could be followed, with difficulty, as far as the temporal lobe.

Three layers can be distinguished in this cortex (Pl. 1, fig. 3; Text-fig. 3); 1: an outer zonal layer containing a few neuroglial cells and some fine myelinated fibres; 2: an intermediate layer of relatively closely packed cells, small and medium in size, and rounded or pyramidal in shape; 3: a deep layer, much thicker than layer 2, containing cells of various shapes, mostly rounded or pyramidal, and larger and less closely packed than those in layer 2. This layer extends deeply as far as the mass of fibres encapsulating the caudate nucleus. Towards the medial side, the cells of layer 2 are larger, and stain more deeply and the layer is more clearly defined. It is therefore possible to distinguish a narrow medial, from a wider lateral part of this cortex.

It is clear that in this cortical area the general characteristics are those of allocortex rather than isocortex. In structure it resembles very closely the *area praepyriformis* which was illustrated by Rose (1926) from a large series of mammals.



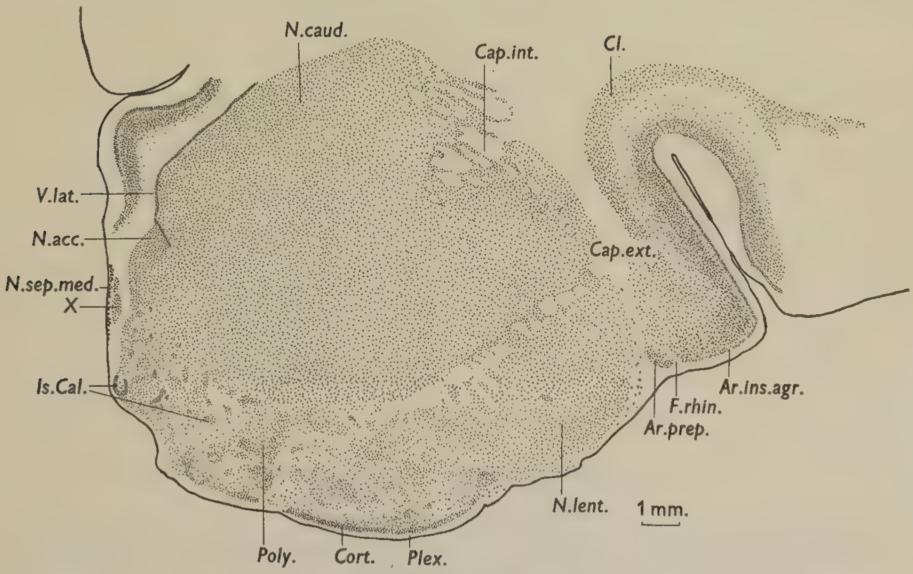
Text-fig. 3. Drawing of a section along the line AA (Text-figs. 2 and 7), to show the prepyriform cortex and its surroundings.

Since it also occupies a similar position in the brain, it is reasonable to conclude that it does in fact represent the prepyriform area of the porpoise brain. Whether its apparent subdivisions are comparable to Rose's *area praepyriformis* 1 and 2, it is impossible to say; subdivisions defined by small differences seen in material of this kind are of very doubtful significance.

Laterally, this cortex is limited for part of its extent by the shallow sulcus labelled *b* in Pl. 1, fig. 1, which should therefore represent the anterior part of the rhinal fissure. Where no depression is visible on the surface, an indentation of lamina 2 can still be seen in section. Beyond this sulcus, or the indentation of lamina 2 when that alone is present, the prepyriform area is continuous with an entirely different type of cortex which has the characteristics of an agranular isocortex (Pl. 1, fig. 4), and which extends laterally on to the operculated area which was compared (p. 98) with the human insula. Five laminae can be distinguished in this cortex, the second of which shows a peculiar arrangement of its cells in clumps. In this and other particulars the cortex in question is very similar to the *area agranularis insularis* defined by Rose (1928) in many mammals, in all of which it is found immediately adjacent to the prepyriform area on its lateral side. The anterior part of the rhinal fissure may therefore be defined as the sulcus marking the line of transition between the prepyriform area and the *area agranularis insularis*, and there seems no doubt that it is represented by the sulcus labelled *b* in the porpoise; this sulcus is poorly developed, however, and the lateral border of the prepyriform area is marked over much of its extent only by the indentation of lamina 2, and by no feature which can be recognized on the surface.

The area désert

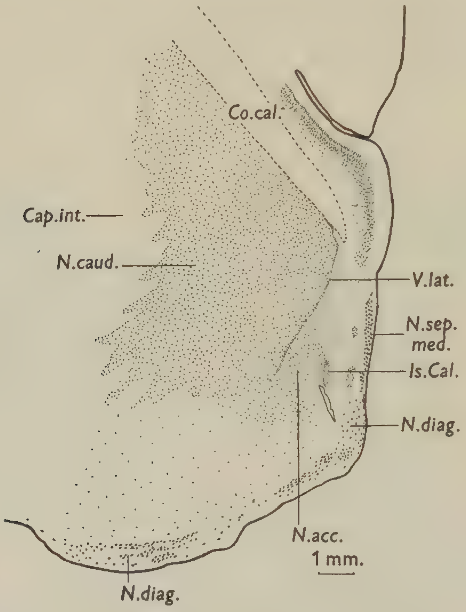
In Nissl preparations four distinct fields can be outlined in this area; their approximate boundaries are shown in Text-fig. 2, and their appearance at different sectional levels in Text-figs. 3, 4, 6 and 10. Anteriorly the caudate nucleus is exposed on the surface (Text-fig. 3), no cortex or other formation intervening except for a narrow layer of myelinated fibres in its anterior part; behind this, is a region in which the characteristic layers of the olfactory tubercle of macrosmatic mammals can be seen (Text-fig. 4), and behind this again, just anterior to the optic tract, a system of fibres with large cells interspersed which appears to represent the diagonal band and its nucleus (Text-fig. 6). The rest of the area, including the part which extends into the Sylvian fossa, is occupied by a mass of myelinated fibres and scattered large cells.



Text-fig. 4. Drawing of a section along the line *BB* (Text-figs. 2 and 7), to show the olfactory tubercle formation.

Clearly the field in which characteristic olfactory tubercle formation is present is of greatest interest. Three layers have been described in the olfactory tubercle of other mammals; an outer plexiform layer, an intermediate cortical or pyramidal cell layer, and an inner broad layer of scattered polymorph cells. In addition, clumps of cells, the 'islets of Calleja', are characteristic of this formation, being found both in the cortical and polymorph layers. Some islets consist of pyramidal cells, others of smaller cells classed as 'granules'.

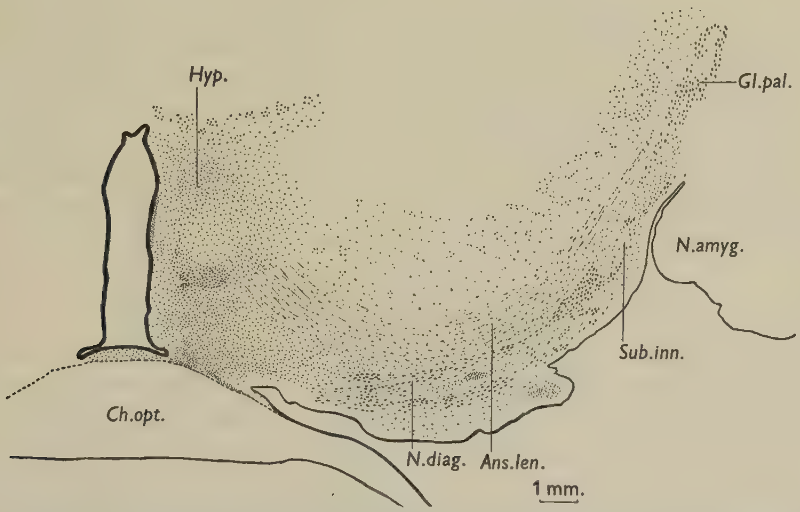
All the above features can be seen in the intermediate region of the area désert in the porpoise (Text-fig. 4). The outer plexiform layer is narrow and contains some myelinated fibres and a few cells which look as if they had migrated from the subjacent layer. The latter, the cortical or pyramidal layer, varies somewhat in development in different parts of the area. When typically developed (Pl. 2, fig. 6), it takes the form of a narrow, slightly undulating, discontinuous layer of small to medium-sized pyramidal cells, although a number of the cells are rounded and a few are small enough to be classified as granules. The layer is best seen postero-laterally when it is continuous for wide stretches in a section (Pl. 2, fig. 11). Medially, where it extends into the precommissural area, it is less definite, more frequently broken, and contains a few islets such as were described by Loo (1931) in the opossum (Pl. 2, fig. 8).



Text-fig. 5. Drawing of a section along the line *CC* (Text-fig. 7), to show the nucleus of the diagonal band.

The deepest or polymorph layer forms a broad zone which in Nissl preparations cannot be distinguished sharply from the underlying caudate nucleus. There is a region of transition which is marked in Weigert preparations by the presence of coarse bundles of myelinated fibres. The cells in this layer vary greatly in type and distribution (Pl. 2, fig. 6). Scattered, large, deeply stained polymorphic cells are characteristic, but medium and small pyramids and granule cells are also found. Islets of both the granular and pyramidal types are present, particularly medially. A prominent granular islet, medial to the nucleus accumbens, seems to be characteristic of many mammals (Loo, 1931; Crosby & Humphrey, 1941), and a similar islet is present in the porpoise (Text-fig. 5). The polymorph layer contains many fibres, running in various directions; posteriorly they tend to form a compact bundle which probably represents part of the medial forebrain bundle.

It can be seen that this formation in the porpoise corresponds in considerable detail with the olfactory tubercle formation of other mammals. The passage from a medial part with a poorly developed cortical layer and many islets, to a postero-lateral part in which islets are absent and the second or cortical layer very clearly developed, suggests comparison with the medial, intermediate and lateral regions defined by Loo in the opossum. It is obvious, however, that the formation as a whole is much less well developed than in macrosmatic mammals, although quite as prominent a feature as the corresponding structure in microsmatic man, and closely similar in detail (Crosby & Humphrey, 1941). The anterior and antero-lateral parts are extremely poorly differentiated, and in places are represented only by scattered masses of cells lying superficial to the striatum.



Text-fig. 6. Drawing of an oblique section along the ventral part of the nucleus of the diagonal band and showing its relationship to the substantia innominata.

Forming a sharp posterior boundary to the olfactory tubercle and separating it from the optic tract is a group of large, deeply staining cells, each with a prominent nucleolus and coarse Nissl granules. These are accompanied by a well-marked group of fibres, the two probably representing the nucleus and tract respectively of the diagonal band. Medially, the former extends around the ventro-medial margin of the hemisphere to become continuous with the medial septal nucleus (Text-fig. 5). Laterally (Text-fig. 6), it comes into relation with groups of large cells similar in character, scattered amongst a mass of fibres occupying the postero-lateral part of the area désert, ventral to the globus pallidus and ansa lenticularis. These fibres, which are lying quite superficially, appear to be emerging from the medial end of the globus pallidus and external capsule, and they are intermingled with the lateral fibres of the diagonal band. The cells associated with them probably belong to the entopeduncular group of nuclei (substantia innominata of Reichert), and their relationship to the lateral end of the nucleus of the diagonal band is similar to that described by Crosby & Humphrey (1941) in man, and by von Bonin (1951) in *Tarsius*. This extensive system of cells and fibres is the formation regarded by Addison (1915) as possibly representing the remnants of the olfactory lobe in the dolphin.

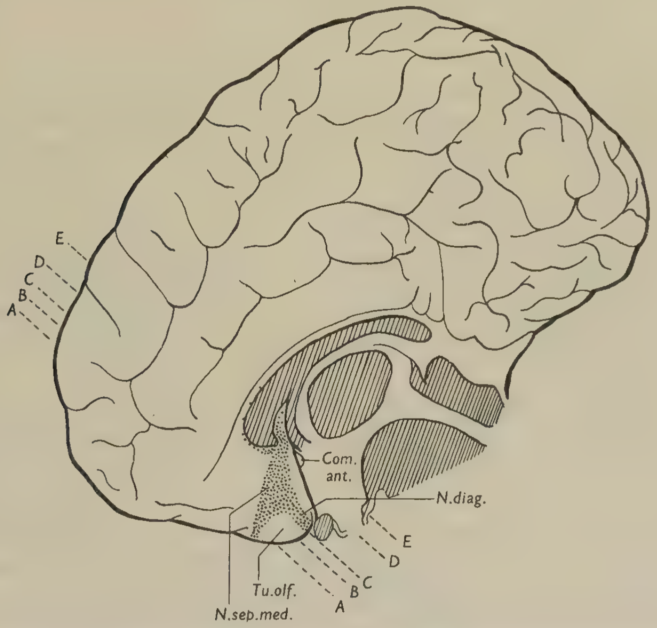
Further anteriorly, cells of a similar type—again accompanied by fibres, which appear to form the lower edge of the external capsule (Text-fig. 10)—are encountered lying superficially in relation to the lateral aspect of the striatum, along the line of the limiting sulcus of the area désert (Text-figs. 3 and 4). They can be followed around the anterior end of the caudate nucleus on to the medial surface where they join the ventral end of the medial septal nucleus, the cells of which are again large and deeply staining. The position of these cells is indicated by the dots along the edge of the caudate nucleus in Text-fig. 2. Thus, it can be seen that the surface area occupied by the corpus striatum and olfactory tubercle is surrounded on all sides by a system of superficial fibres accompanied by large cells of characteristic appearance. Medially (Text-fig. 7) they form the medial septal nucleus and nucleus of the diagonal band; posteriorly, they form the continuation of the latter and the substantia innominata, while anteriorly and laterally are the cells described above (Text-fig. 2). The fact that the cells are all of a similar type, and are scattered in fibre systems which appear to be closely related to the corpus striatum, suggests a common function. No more detailed analysis is possible in this material, however, so that a more definite statement cannot be made.

The precommissural area and septum pellucidum

In the precommissural region of mammals many nuclear cell groups have been identified. In general these are ill defined, and consist of cells scattered along the course of the fibre systems found in this situation. These fibre systems are themselves complex and the precise connexions and the direction of conduction of their various components are very little known. For these reasons, the terminology used in the description of the precommissural structures can do no more than indicate the position and relations of cell groups, the outlines of which are for the most part indefinite, and the precise connexions of which are not known. These general considerations apply to the precommissural region of the porpoise, and in the description which follows, the terms applied to the different cell groups have neither more nor less significance than when applied in other mammals.

Medial septal nucleus

This is the most striking feature of the precommissural area. It has already been mentioned in connexion with the nucleus of the diagonal band and the system of fibres and cells which surround the striatum and olfactory tubercle. Throughout, its cells are of the same type, large, with coarse Nissl granules, and generally rounded or multipolar in shape (Pl. 2, fig. 9). Characteristically, it forms a narrow plate of cells immediately beneath the surface, lying among the fibres in this situation (Text-figs. 3, 4 and 8). Its entire extent is illustrated in Text-fig. 7. It is separated from the cortex on the medial side of the frontal lobe by a cell-free interval, although, here and there, outlying cells, similar to those in the main part of the nucleus, can be found in the zonal layer of the cortex, and also in the fibres deep to the cortical laminae. Posteriorly and dorsally, it extends into the septum pellucidum above the anterior commissure (Text-fig. 9) and disappears at a level just in front of the interventricular foramen. In this region it appears to establish continuity with similar cells scattered among the fibres of the fornix. Its ventral boundary is indistinguishable posteriorly from the nucleus of the diagonal band (Text-fig. 5), and anteriorly, is continued as the scattered large cells previously described as lying in front of the anterior margin of the caudate nucleus on the ventral surface; between these two situations, the ventral boundary comes into relation with the medial part of the olfactory tubercle.



Text-fig. 7. Drawing of a median sagittal section of the brain on which the extent of the medial septal nucleus is shown. The lines *AA*, etc., indicate the planes of section of Text-figs. 3, 4, 5, 8 and 9, in that order from front to back. Natural size.

Immediately anterior to the genu of the corpus callosum there is a small group of cells, still of the same type, but separated from the main part of the medial septal nucleus by a narrow cell-free interval (Text-fig. 8). Cells which apparently belong to this same group can be found extending posteriorly for a short distance on the ventral aspect of the genu and also above the genu, where they are marked Z in Text-fig. 8, on to the dorsal aspect of the corpus callosum to become intermingled with the fibres of a well-marked medial stria of *Lancisius*. This probably represents the 'true induseum' of Elliot Smith (1897) which he derived from the commissure bed, and contrasted with a more laterally situated 'false induseum' formed by the hippocampal vestiges. Humphrey (1936, fig. 6) illustrates a similar group of cells in the bat.

In its extent and general relations this medial septal nucleus corresponds very closely with the structure similarly named in other mammals. Its cells are relatively larger and more conspicuous in the porpoise; the indefiniteness of its boundaries might cause some uncertainty whether outlying groups of cells should be included in it or given a separate name, but the same difficulty is met with in mammals generally, so that it does not invalidate the comparison.

The lateral septal nucleus

This is less definite than the medial. It consists of a mass of cells, very varied in shape, mostly small rounded or fusiform (Pl. 2, fig. 10), which occupies the septum pellucidum lateral to the medial septal nucleus (Text-fig. 9). It is traversed by the more laterally situated fibres of the precommissural fornix system and blends ventrally with the bed nuclei of the anterior commissure and stria terminalis. In the transition region between the septum pellucidum and the precommissural area it is found lying dorso-medial to the nucleus accumbens, and in this situation it is difficult to define the exact boundary between the two.

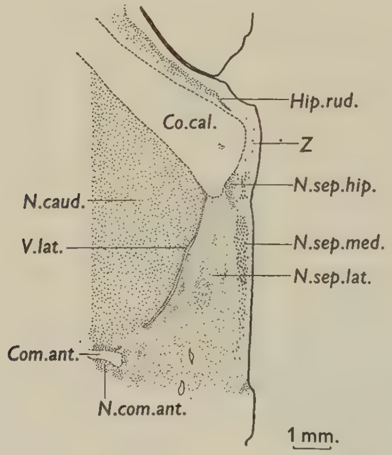


Fig. 8.

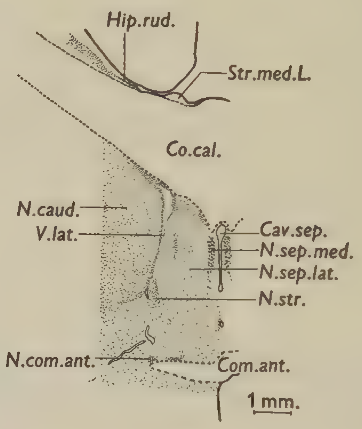


Fig. 9.

Text-fig. 8. Drawing of a section just in front of the genu along the line *DD* (Text-fig. 7). *Z*, cells ascending above the genu to become continuous with the 'true induseum'.

Text-fig. 9. Drawing of a section along the line *EE* (Text-fig. 7).

Further anteriorly, several small clumps of cells may be found lying in, and encapsulated by, the fibre stratum between the medial septal nucleus and nucleus accumbens (*X*, Text-figs. 4 and 10); from their position, they might be regarded as representing an anterior part of the lateral septal nucleus, but as the cells are similar in character to those of the nucleus accumbens, they should probably be classed with it. Jelgersma (1934) illustrates similar groups, but does not comment upon them.

The nucleus accumbens itself is large. As in other mammals, it is formed by an extension of the caudate nucleus round the lower limit of the lateral ventricle into the precommissural area. Its identity of histological structure with the caudate nucleus makes it doubtful whether a separate name should be used to describe it, but this is the common practice.

Anterior hippocampal cortex

This comprises the edge of the medial cortex immediately beyond the precommissural area in mammals. While extensive and well differentiated in the monotremes and marsupials, this cortex is vestigial in placental mammals. Throughout the greater part of its extent in the porpoise, the margin of the cortex related to the precommissural area differs from the general isocortex only in its relative narrowness and in the absence of typical lamination. It is doubtful whether it can be regarded as representing a typical anterior hippocampal cortex. However, just anterior to the genu of the corpus callosum it undergoes a characteristic differentiation. The cells become spindle-shaped and stain more deeply, forming a narrow band which can be followed above the genu (Pl. 2, fig. 5) into continuity with what is undoubtedly a supra-callosal hippocampal rudiment (Pl. 2, fig. 7), the 'false induseum' of Elliot Smith (1897). There seems little doubt that this narrow strip of cells represents the anterior hippocampal cortex of the porpoise.

Young (1936) in the rabbit, and Humphrey (1936) in the bat, describe as a 'septo-hippocampal nucleus' a posterior extension of the anterior hippocampal cortex, ventral to the genu. In Text-fig. 8, a group of cells can be seen to occupy a similar position in the porpoise, and as it can be traced forwards into continuity with the anterior hippocampal cortex, it probably corresponds with the nucleus described by Young and Humphrey. The necessity for giving it a separate name is questionable.

In general, it can be said of the precommissural area of the porpoise that representatives of all the more important cell groups found here in other mammals are present and recognizable. There are differences in detail and in proportions, but the general pattern is the same.

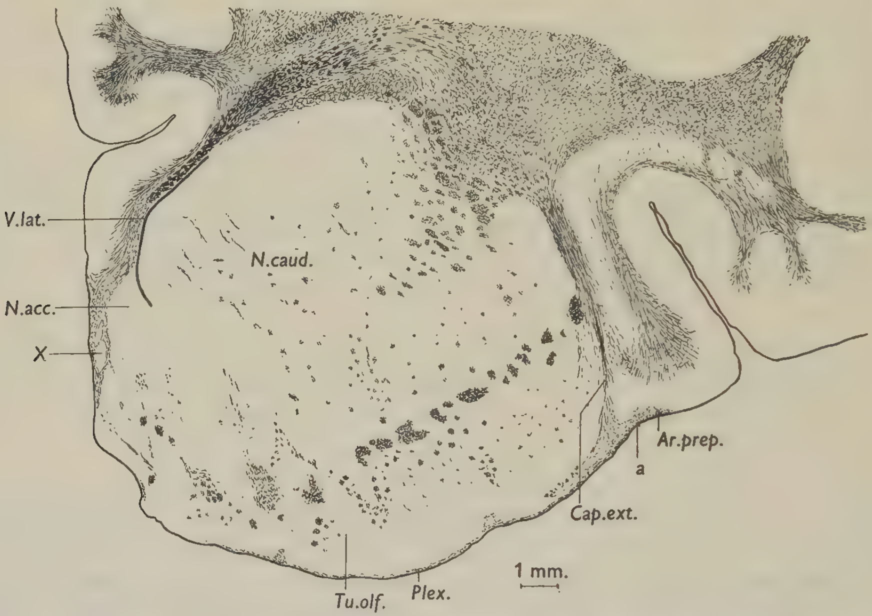
Fibre systems

Fibres of the precommissural area. Just beneath the surface is a mass of well-myelinated fibres in which the medial septal nucleus is embedded (Text-fig. 10). The deeper fibres of this system form a capsule around the nucleus accumbens, and the more superficial appear to be fibres of passage, traversing the region on their way to other centres. Many of the latter sweep around the ventro-medial margin to form a well-defined diagonal band.

Above the corpus callosum and close to the midline, is a prominent bundle which is apparently the medial stria of *Lancisius* (Text-fig. 9). Embedded in it are a few scattered cells previously mentioned as representing the 'true induseum' of Elliot Smith. More laterally, fibres can be seen in the superficial part of the hippocampal rudiment, and these appear to enter the stria along its course. Followed anteriorly, both sets of fibres enter the precommissural area in front of the genu, and, although most become lost in the general mass of fibres here, a distinct bundle can be traced, just below the surface, into the diagonal band.

Occupying the septum pellucidum is a mass of fibres which appears to emerge from the fornix. Traced anteriorly, they enter the precommissural area, traversing on their way the lateral and medial septal nuclei. In front of the anterior commissure they spread out, and can be traced with varying degrees of difficulty in two main directions: some appear to enter the diagonal band, and others turn posteriorly beneath the anterior commissure and proceed towards the preoptic region. This system of fibres would appear to represent the precommissural fornix, which is apparently quite well developed in the porpoise.

Fibres related to the area désert. Some reference has already been made to these in describing the cellular pattern of the area, and little can be added here. Unfortunately, nothing can be said concerning the origin of the fibres in the zonal layer of the prepyriform cortex, nor of those in the plexiform layer of the olfactory tubercle. The fact that they are present, however, is of considerable interest, as in other mammals such fibres are apparently derived from the lateral and intermediate olfactory tracts respectively.



Text-fig. 10. Drawing of a Weigert section 40μ posterior to that from which Text-fig. 4 was made.

Anterior commissure. This is just visible to the naked eye, and is quite obvious on microscopic examination (Text-fig. 9). Jelgersma (1934) failed to identify it in his material. From the mid-line, where it is in direct relation posteriorly with the anterior column of the fornix, it can be traced laterally and ventrally for some distance, to become lost in a mass of fibres ventral to the corpus striatum. There is no clear-cut indication of a subdivision into temporal and olfactory components as in other mammals.

Portions of the fornix, stria semicircularis, and stria habenularis were also encountered, but a description of these will be referred to a future paper, when they can be described in their entirety. So far as they entered the field of the present investigation, they did not appear to differ significantly from the same systems in other mammals.

From the above rather limited description, it can be seen that, with the exception of the olfactory tracts, representatives of the main fibre systems usually associated with the parts of the rhinencephalon described in this paper, are present in the porpoise.

DISCUSSION

The rhinal fissure and the nature of the operculated cortex. On the basis of the identification of a prepyriform cortex (p. 100), the probable position of the rhinal fissure in *Phocaena* has been identified (see Text-fig. 2). On the same grounds, and by a comparison of the appearances found with those illustrated by Rose (1928), the cortex immediately lateral to it, as well as that of the operculated area, has been identified as insular cortex. This interpretation differs considerably from that advanced by Ries & Langworthy (1937). They state that 'it is the rhinal fissure which in Cetaceans forms the fissure surrounding the insula. The opercular region...is all a portion of the rhinencephalon, corresponding to the lateral olfactory gyrus'. This interpretation was based upon Anthony's (1925) study of the fissures of an embryo of *Megaptera boops*, but confirmatory histological evidence was not presented. If correct, it would lead to the rather extraordinary conclusion that in the Cetacea, which are either anosmatic, or extremely microsmatic, the lateral olfactory gyrus is enormously developed to a size greater even than that found in macrosmatic mammals. This is most unlikely, and the operculated area should be regarded as belonging to the insula, especially as it shows the characteristics of isocortex, and lies superficial to a typical claustrum. It is not suggested that this area is homologous with the entire insula of man—as indeed, it would appear not to be, since the claustrum extends considerably beyond its limits (Text-fig. 4)—but as this question is outside the scope of the present work, it will not be considered further.

Functional considerations. The interest of the study of an anosmatic brain lies largely in the light it may throw on the functions of those parts of the brain which are thought to be concerned with olfaction. It is generally assumed that if a structure loses its function it is likely to atrophy or even disappear and that its failure to disappear, even when its presumed function is lost, is evidence that it must be concerned with some other function than that originally postulated for it. It has been suggested, for example (Jelgersma, 1934), that the persistence of a hippocampal formation in the anosmatic brain is evidence that the hippocampus has other than olfactory functions, not only in the anosmatic brain under consideration, but in mammals generally.

In the porpoise it is clear that no olfactory function in the ordinary sense can exist, since the olfactory nerve, bulbs and tracts are not present, and it is equally clear that this is a secondary, and not a primary, condition. There is no reason to believe that the terrestrial mammals from which the Cetacea were derived were anosmatic, and in fact, many of the Cetacea (especially the Mystaceti) still possess small olfactory bulbs and tracts. The ancestors of the porpoise possessed, therefore, not only olfactory bulbs and tracts, but, it may fairly be assumed, other parts of the forebrain necessary for the function of olfaction to be effective.

Investigations like the present, which are limited to purely structural features, do not of course give any direct evidence concerning function. What has been described here are the structural differences found in certain parts of the porpoise brain as compared with the same parts of other mammals; since the parts under consideration have all been thought, with greater or less justification, to receive connexions from the olfactory bulbs, it is a fair presumption that the differences observed may be related to, and perhaps, the result of their loss. Conversely, if parts of the porpoise brain show no structural differences from similar parts in the brains of anosmatic mammals, it may also be assumed that the presence or absence of connexions from the olfactory bulb is immaterial so far as the development and differentiation of these particular structures are concerned.

In the porpoise brain it is clear that the nucleus accumbens, the nuclei of the precommissural area, and the nucleus of the diagonal band are at least as well developed and as highly differentiated as in any other mammal. One gets the impression, in fact, that the nucleus accumbens is relatively larger, and that the medial septal nucleus and the nucleus of the diagonal band are more clearly differentiated. It may be concluded that the presence of secondary olfactory connexions is not necessary for the structural development and differentiation of these nuclei, at least so far as that structure can be revealed by Nissl and Weigert preparations.

The prepyriform cortex and the olfactory tubercle, however, are not quite similar to these structures in other mammals, and an anterior olfactory nucleus has not been identified in the porpoise, although it is possible that part of it may be represented in the antero-medial part of the prepyriform cortex, as in other mammals the two cannot be clearly differentiated anteriorly.

The prepyriform cortex of the porpoise is certainly relatively, and perhaps absolutely, smaller than in most mammals, but its degree of differentiation in Nissl preparations is not conspicuously different. The characteristic olfactory tubercle formation, although present in the porpoise, is less highly differentiated than in macrosmatic mammals, and therefore, not so easy to recognize, and does not cover the whole of the ventral surface of the head of the caudate nucleus; in its anterior and lateral parts it is so poorly developed as to be barely recognizable.

Both prepyriform cortex and olfactory tubercle appear, however, to be quite as well developed as in microsmatic man. It is therefore not possible to say that the presence of connexions from the olfactory bulb is essential for their development, but it is possible, and indeed probable, that the presence of such connexions is essential for their full development and differentiation as seen in macrosmatic mammals.

These findings are consistent with the experimental results of Le Gros Clark & Meyer (1947) and Meyer & Allison (1949). These workers found that so far as the structures under investigation in the porpoise are concerned, the prepyriform cortex, and the anterior and lateral parts of the olfactory tubercle were the only ones in which evidence for the termination of fibres from the olfactory bulbs could be found in the rabbit and in the monkey. Crosby & Humphrey (1941) have also suggested, on comparative anatomical grounds, that there is a correlation between the size of the olfactory bulb and the degree of development of the anterior part of the tubercle.

The present findings, therefore, could be interpreted functionally as follows: Because they show no retrogressive changes in the anosmatic porpoise, the nuclei of the precommissural area, nucleus accumbens, and the nucleus of the diagonal band have no olfactory function in any mammal. The antero-lateral part of the olfactory tubercle is primarily an olfactory nucleus, and has no other important functions; olfactory tubercle formation, as such, may however perform non-olfactory functions, since it is present in the porpoise, but only in a region which receives no direct fibres from the olfactory tracts in other mammals. The prepyriform cortex is also an important olfactory centre, but is capable of performing other functions since it persists, but in a reduced form, in the porpoise.

These interpretations are quite possibly correct, but they are of a different order of reliability from the statement, for example, that connexions from the olfactory bulbs are not essential for the full development and differentiation of the precommissural nuclei. The fact that direct olfactory tract fibres have not been shown to reach the posterior part of the olfactory tubercle is not evidence that it has no olfactory function, as was suggested by Meyer & Allison (1949), who divided the tubercle into olfactory and non-olfactory areas. The visual cortex does not receive fibres direct from the retina, but its importance for visual function is undoubted. Until much more is known of tertiary and other olfactory connexions within the forebrain, it is impossible to generalize, on the basis of purely structural investigations, on the presence or absence of olfactory function in particular regions. It is probable, in the past, that olfactory functions have been distributed too widely throughout a hypothetical 'rhinencephalon'; it is only recently that we have begun to acquire the knowledge on which more precise statements can be based, but it is important not to assume a stricter limitation of olfactory function within the forebrain than the present state of that knowledge would really justify.

SUMMARY

Despite the absence of olfactory bulbs and tracts, the olfactory tubercle, prepyriform cortex, nucleus of the diagonal band, and the nuclei of the precommissural region, or septum, can be identified in the brain of the porpoise; an anterior olfactory nucleus, as such, could not be defined.

The anterior and antero-lateral parts of the olfactory tubercle are very poorly differentiated, but the cellular pattern of the remainder is very similar to that of the corresponding formation in other mammals; the prepyriform cortex is poorly developed, but can be recognized as a narrow strip lying medial to an ill-defined rhinal fissure; the nuclei of the precommissural region and the nucleus of the diagonal band are as well developed, and, with the possible exception of the lateral septal nucleus, show as high a degree of differentiation as do the corresponding nuclei in macrosmatic mammals.

On the basis of these findings it is concluded that with the possible exception of the anterior olfactory nucleus and the antero-lateral part of the olfactory tubercle, the presence of secondary olfactory connexions is not essential for the development and differentiation of these structures. The implications arising from this conclusion are discussed from a functional point of view.

The material for this study was provided through the kindness and co-operation of Dr E. Ford, Director of the Marine Station, Millport, Isle of Cumbrae. My thanks are due to Prof. F. Goldby for helpful advice and criticism throughout, and to Mr R. Jarrett who prepared the sections and most of the photomicrographs, and to the Photographic Department, St Mary's Hospital Medical School, for figures 1 and 2 in Pl. 1.

REFERENCES

Addison, W. H. F. (1915). On the rhinencephalon of *Delphinus delphis*, L. *J. comp. Neurol.* **25**, 497–522.

Anthony, R. (1925). Sur un cerveau de Mégaptère. *C.R. Acad. Sci., Paris*, **181**, 681–683.

von Bonin, G. (1951). The isocortex of *Tarsius*. *J. comp. Neurol.* **95**, 387–428.

Brodal, A. (1947). The hippocampus and the sense of smell. *Brain*, **70**, 179–222.

Crosby, E. C. & Humphrey, T. (1941). Studies of the vertebrate telencephalon. II. The nuclear pattern of the anterior olfactory nucleus, tuberculum olfactorium, and the amygdaloid complex in adult man. *J. comp. Neurol.* **74**, 309–352.

Elliot Smith, G. (1897). The relation of the fornix to the margin of the cerebral cortex. *J. Anat., Lond.*, **32** (N.S. no. 12), 23–58.

Humphrey, T. (1936). The telencephalon of the bat. *J. comp. Neurol.* **65**, 603–711.

Jelgersma, G. (1934). *Das Gehirn der Wassersäugetiere*. Leipzig: Johann Ambrosius Barth.

Kükenthal, W. & Ziehen, T. (1889). Vergleichend-anatomische und Entwickelungsgeschichtliche Untersuchungen an Walthieren. Kapitel III. Das centralnervensystem der Cetaceen. *Denkschr. med.-naturw. Ges. Jena*, Bd. **3**, Teil 1, 80–200.

Langworthy, O. R. (1932). A description of the central nervous system of the porpoise (*Tursiops truncatus*). *J. comp. Neurol.* **54**, 437–488.

Le Gros Clark, W. E. & Meyer, M. (1947). The terminal connexions of the olfactory tract in the rabbit. *Brain*, **70**, 304–328.

Loo, Y. T. (1931). The forebrain of the opossum, *Didelphis virginiana*. II. Histology. *J. comp. Neurol.* **52**, 1–148.

Meyer, M. & Allison, A. C. (1949). An experimental investigation of the connexions of the olfactory tracts in the monkey. *J. Neurol. Psychiat.* **12**, N.S., 274–286.

Ries, F. A. & Langworthy, O. R. (1937). A study of the surface structure of the brain of the whale (*Balaenoptera physalis* and *Physeter catodon*). *J. comp. Neurol.* **68**, 1–48.

Rose, M. (1926). Der Allocortex bei Tier und Mensch. *J. Psychol. Neurol. Lpz.*, Bd. **34**, Heft 1, 1–111.

Rose, M. (1928). Die Inselrinde des Menschen und der Tiere. *J. Psychol. Neurol. Lpz.*, Bd. **37**, 467–624.

Thompson, I. McL. (1932). On the cavum septi pellucidi. *J. Anat., Lond.*, **67**, 59–77.

Wilson, R. B. (1933). The anatomy of the brain of the Whale (*Balaenoptera sulfurea*). *J. comp. Neurol.* **58**, 419–480.

Young, M. W. (1936). The nuclear pattern and fiber connections of the non-cortical centers of the telencephalon of the rabbit (*Lepus cuniculus*). *J. comp. Neurol.* **65**, 295–401.

EXPLANATION OF PLATES

All photographs are of the brain of *Phocaena phocaena*; figs. 3–10 are of celloidin sections 20μ thick, stained with thionin.

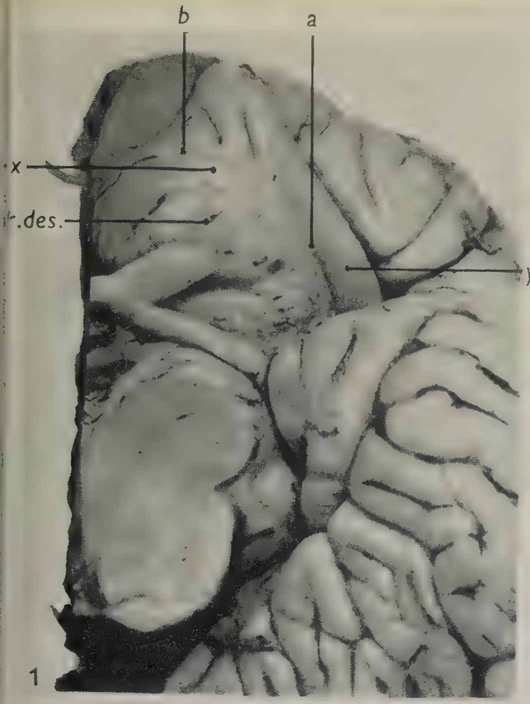
Plate 1

Fig. 1. Ventral aspect of the left hemisphere showing the area désert; adjacent gyri and sulci marked *a*, *b*, *X* and *Y* are described on p. 97 of text. Natural size.

Fig. 2. Part of the medial aspect of the right hemisphere showing the precommissural area and its relations. The brain was cut slightly to the left of the midline, so that the hemisphere shown includes both leaves of the septum pellucidum. × 2.

Fig. 3. The prepyriform cortex, showing its three layers, marked 1, 2 and 3. × 15.

Fig. 4. The cortex of the area agranularis insularis. × 32.



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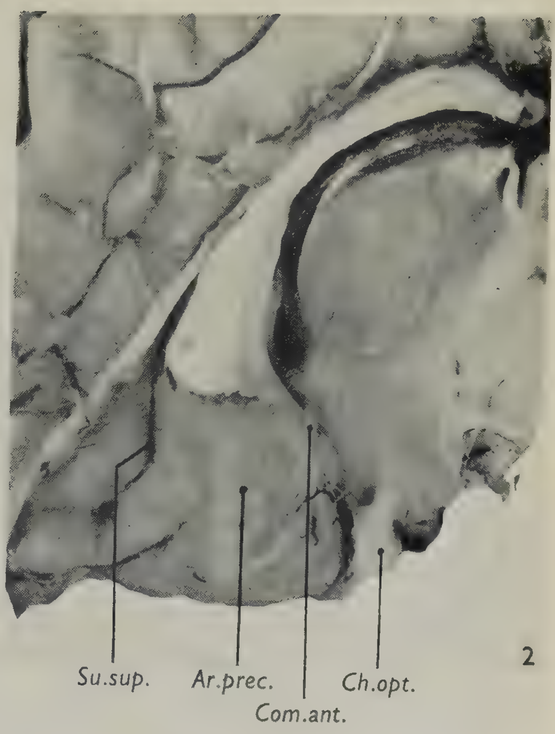

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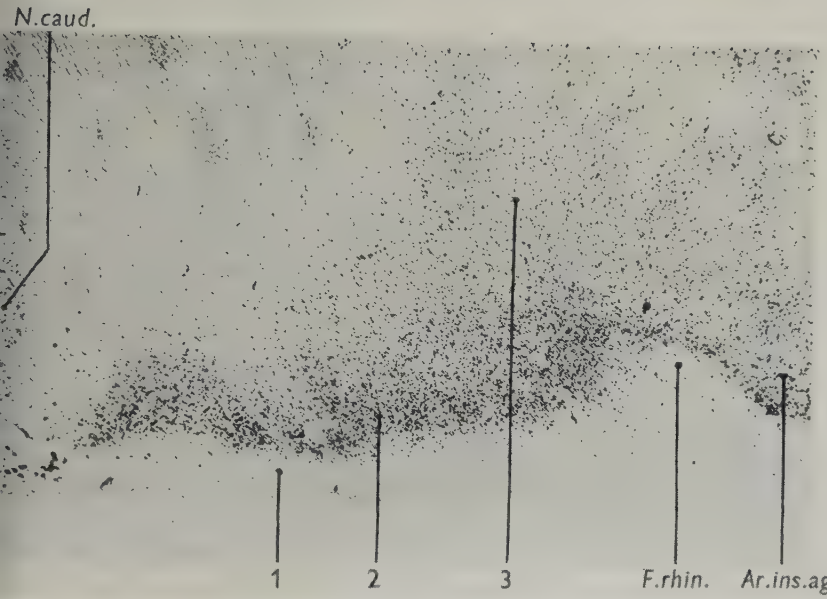

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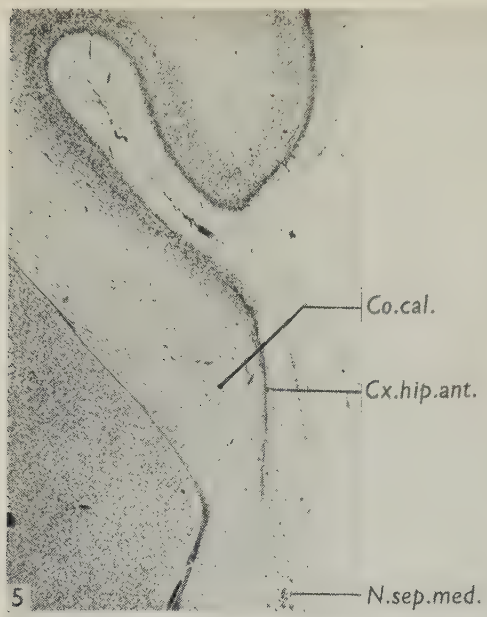
EATHNACH—The olfactory tubercle etc., of the porpoise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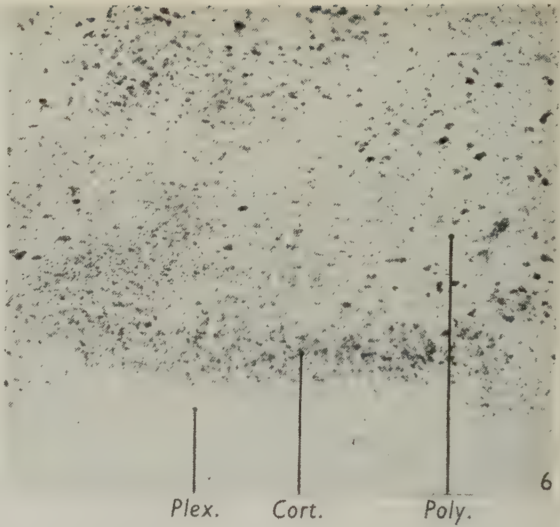
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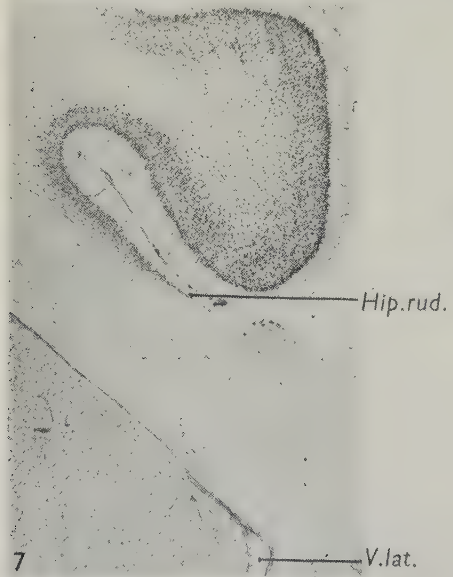
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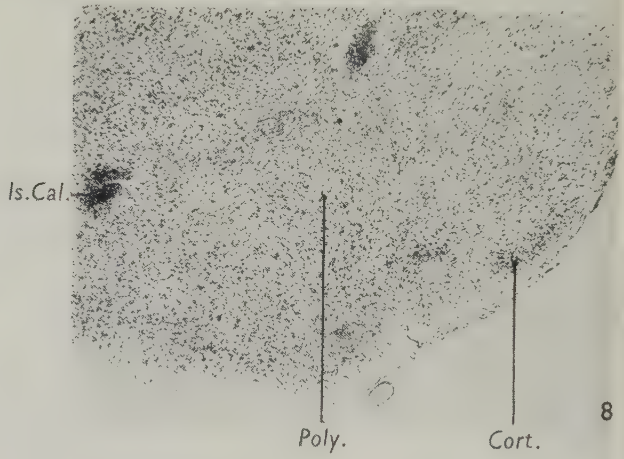
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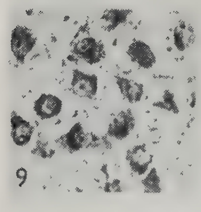
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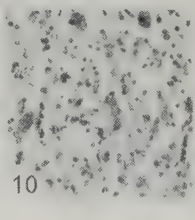
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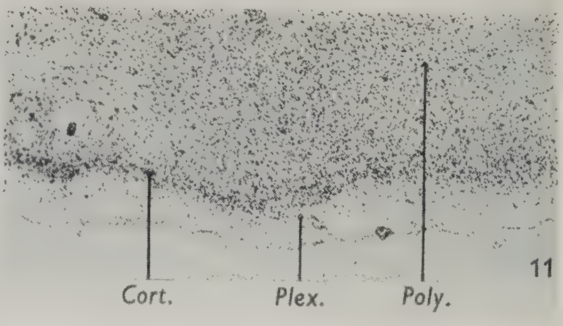
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BREATHNACH—The olfactory tubercle etc., of the porpoise

Plate 2

Fig. 5. Oblique section, just in front of the genu of the corpus callosum, showing the anterior hippocampal cortex. ×10.

Fig. 6. Transverse section showing the three layers of the olfactory tubercle. ×47.

Fig. 7. Oblique section through the anterior part of the corpus callosum showing the supra-callosal hippocampal rudiment. ×10.

Fig. 8. A section through the medial part of the olfactory tubercle adjacent to the precommissural area. ×20.

Fig. 9. Cells of the medial septal nucleus. ×126.

Fig. 10. Cells of the lateral septal nucleus. ×126.

Fig. 11. The postero-lateral part of the olfactory tubercle. ×20.

KEY TO ABBREVIATIONS

Single letters are used in some of the figures to indicate unnamed structures which are described in the text.

Ans.len.	Ansa lenticularis
Ar.des.	Area désert
Ar.ins.agr.	Area insularis agranularis
Ar.prec.	Precommissural area
Ar.prep.	Prepyriform area
Cap.ext.	External capsule
Cap.int.	Internal capsule
Cav.sep.	Cavum septi pellucidi
Ch.opt.	Optic chiasma
Cl.	Claustrum
Com.ant.	Anterior commissure
Co.cal.	Corpus callosum
Cort.	Cortical or pyramidal layer of olfactory tubercle
Cx.hip.ant.	Anterior hippocampal cortex
Cx.oper.	Operculated cortex
F.rhin.	Rhinal fissure
For.	Fornix
Fos.syl.	Sylvian fossa
Gl.pal.	Globus pallidus
Hip.rud.	Supra-callosal hippocampal rudiment
Hyp.	Hypothalamus
Is.Cal.	Islet of Calleja
N.acc.	Nucleus accumbens
N.amyg.	Amygdaloid nucleus
N.caud.	Caudate nucleus
N.com.ant.	Bed nucleus of anterior commissure
N.diag.	Nucleus of diagonal band
N.lent.	Lentiform nucleus
N.sep.hip.	Septo-hippocampal nucleus
N.sep.lat.	Lateral septal nucleus
N.sep.med.	Medial septal nucleus
N.str.	Bed nucleus of stria terminalis
Plex.	Plexiform layer of olfactory tubercle
Poly.	Polymorph layer of olfactory tubercle
Str.med.L.	Medial stria of Lancisius
Su.sup.	Supra-callosal sulcus
Sub.inn.	Substantia innominata of Reichert
Tu.olf.	Olfactory tubercle
V.lat.	Lateral ventricle

VASCULAR PATTERNS IN THE CHOROID PLEXUS

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The central position in the field of research into the circulation of the cerebrospinal fluid has long been occupied by the controversy as to whether the fluid is a filtrate or a secretion. The evidence on both sides has been comprehensively reviewed by Weed (1922) and Flexner (1933). In the presence of this controversy, other problems which may be germane to the whole question of the production of the cerebrospinal fluid have been largely overlooked.

Although it is generally accepted at the present time that the choroid plexuses are the main source of the cerebrospinal fluid, little is to be found in the literature concerning the blood vessels of these plexuses. Neither Weed nor Flexner make any reference to the arrangement of the choroidal vessels or to the presence or absence of arteriovenous anastomoses in the plexuses.

The experiments of Dandy (1919) and the criticism to which his findings have been subjected by Flexner (1933) have shown the difficulties which beset attempts to draw conclusions as to the nature and method of production of the cerebrospinal fluid from experimental operations upon the choroid plexuses. The situation and relationships of the plexus must always make the performance of such physiological experiments difficult and the interpretation of the results uncertain and inconclusive.

The work of Trueta, Barclay, Daniel, Franklin & Prichard (1947) has shown that fresh light may be thrown upon a problem, which direct experimentation appears unlikely to solve, by a study of the vascular patterns of the organs concerned. It has appeared therefore that the nature of the vascular patterns of the choroid plexuses may be equally pertinent to the question of the production of the cerebrospinal fluid. An examination of the literature has failed to disclose any description of the general arrangement of the blood vessels of the choroid plexus, and this has encouraged the study of whole cleared preparations of injected plexuses as being fundamental to the further investigation of the possible control of the production of the cerebrospinal fluid by nervous or other mechanisms.

In the present study attention has been confined to the choroid plexuses of the lateral ventricles, as these have been the subject of most physiological investigation and are usually considered to be the main source of production of the cerebrospinal fluid.

MATERIALS AND METHODS

In this investigation the brains of fourteen rabbits, four human foetuses and twelve human adults were examined. The rabbits were injected immediately after death with coloured substances into the common carotid or brachiocephalic arteries. The whole head was then fixed in 10% formalin for 4 days, after which the choroid plexuses of both the lateral and third ventricles were removed entire and cleared before mounting in balsam. The human foetuses were of varying periods of gestation. Injection was made through the common carotid arteries of both sides, and the subsequent treatment was similar to that followed in the rabbits. The adult human brains were obtained from the post-mortem room as fresh as possible. They were injected directly into the anterior choroidal artery of each side. The whole brain was then fixed for 4 days in 10 % formalin, and at the end of this period the choroid plexuses were dissected out. After further fixation, the plexuses were dehydrated through graded alcohols, cleared in xylol, and preserved for examination in methyl salicylate.

Various injection media were employed—indian ink in plasma, colloidal carbon, and red and blue dispersion media.* The blue and red dispersion media employed were those normally used for colouring neoprene latex. They were prepared by ball-milling pigments with a small quantity of a highly active surface wetting agent. The blue pigment used was copper phthalocyanine, and the red was a dispersion of a red lake pigment. It was found that both the carbon suspensions and the red dispersion medium passed rapidly over to the venous side of the plexus and gave a complete picture of the venous pattern, but little if any remained on the arterial side. On the other hand, the blue dispersion under the pressures employed tended to remain on the arterial side and outlined fully the arteries and arterioles. The larger veins in these latter specimens could usually be identified by the presence of contained blood. It was decided therefore to inject the red dispersion from the arterial side and to follow this immediately by an injection of the blue dispersion. This red-blue method was found to give a very satisfactory injection of both the arterial pattern (blue) and the venous pattern (red). There was little mixing of the dispersions, and the junction of the blue and red dispersions at the arteriovenous connexions in the fronds was clearly distinguishable.

OBSERVATIONS

In the descriptions of the choroid plexuses both in the rabbit and in man, the convention is adopted throughout of considering the plexus as though it was situated in a single plane. Thus, the part of the plexus occupying the most anterior part of the inferior horn of the ventricle is described as the posterior end of the plexus, and the superior surface is taken as the whole of that surface which is continuous with the superior surface in the body of the ventricle.

The choroid plexus of the rabbit (Text-fig. 1)

In the rabbit the elaborate folding into villous processes of the ependymal layer covering the choroid plexus is restricted to a limited area of the free margin of the plexus. It is most marked anteriorly, near the interventricular foramen, where the free border shows a marked forward angulation. Behind this, at a point corresponding roughly to the trigone of the ventricle, a curious tongue-like process projects laterally. The name of *lingula* is suggested for this process. This area is not an example of villous thickening of the plexus. It is not covered with folded layers of ependyma, and its vascular relations differ from those of the anterior part of the plexus (Pl. 1, fig. 1). In some plexuses there is a second projection posterior to the lingula.

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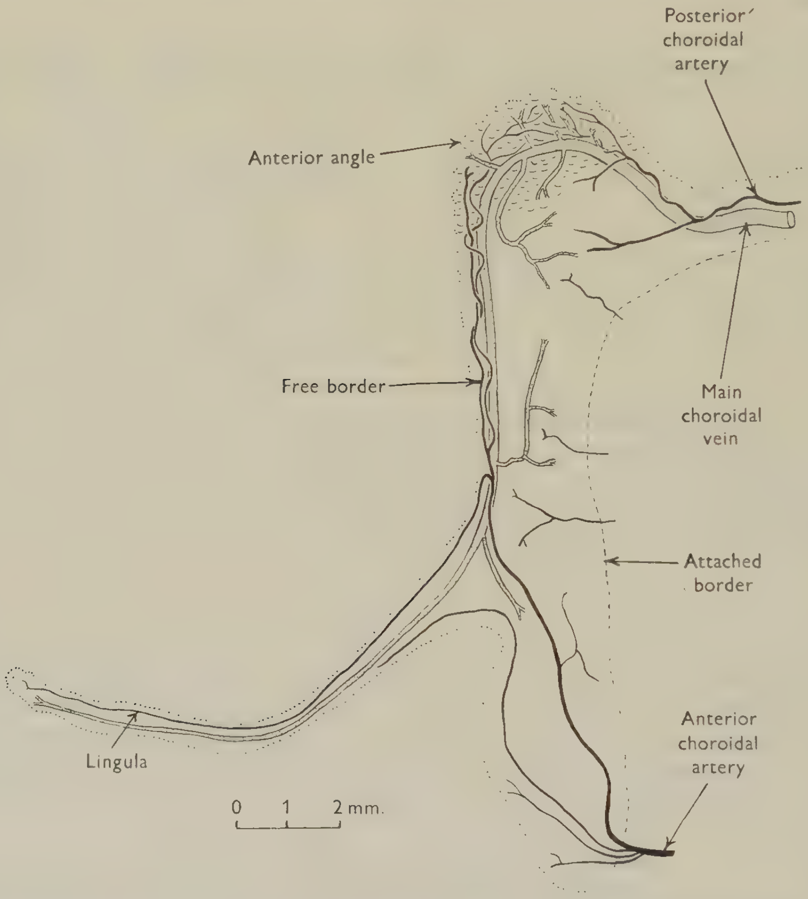
* The dispersion media were obtained from the B.B. Chemical Co., Ltd., Ulverscroft Road, Leicester.

The anterior choroidal artery enters the inferior horn of the ventricle and divides usually into two main branches, one of which runs along the free and the other along the attached border of the choroid plexus (Pl. 1, fig. 2). The branch along the free border continues along this border to the posterior aspect of the lingula where it ends. It is often accompanied in its course by another branch of the parent stem which is very convoluted. The branch along the attached border gives off short stems which divide immediately into branches which pass anteriorly and posteriorly in the attached margin of the plexus. Just after it passes beyond the lingula this branch gives off a recurrent branch to the process which runs along the anterior border of the lingula. Having given off the branch to the lingula, the main branch reaches the free border of the plexus. As it approaches the anterior end where the villous processes are most numerous, it becomes more convoluted and tortuous and ends by becoming continuous with one of the posterior choroidal arteries (Pl. 1, fig. 3).



Text-fig. 1. Diagram to illustrate the principal features of the choroid plexus of the rabbit. (Lateral ventricle: left side.)

There are generally two to four posterior choroidal arteries, branches of the posterior cerebral artery. One thick branch enters the plexus at the interventricular foramen and runs along the free margin of the plexus towards the angle of the free border, giving off in its course a branch to the attached border of the plexus and ends by becoming continuous with the anterior choroidal artery (Pl. 1, fig. 3). A more posterior branch from the posterior cerebral artery frequently runs forwards in the attached border. The main choroidal vein starts at the anterior end of the plexus and runs forwards, gaining tributaries as it progresses towards the interventricular foramen (Pl. 1, fig. 4). It is continuous with the venous network which forms a vein or series of veins leaving the posterior end of the plexus and accompanying the anterior choroidal artery. A constant large vein runs in the free margin of the plexus, and joins the main choroidal vein anteriorly.

The choroid plexus of man (Text-fig. 2)

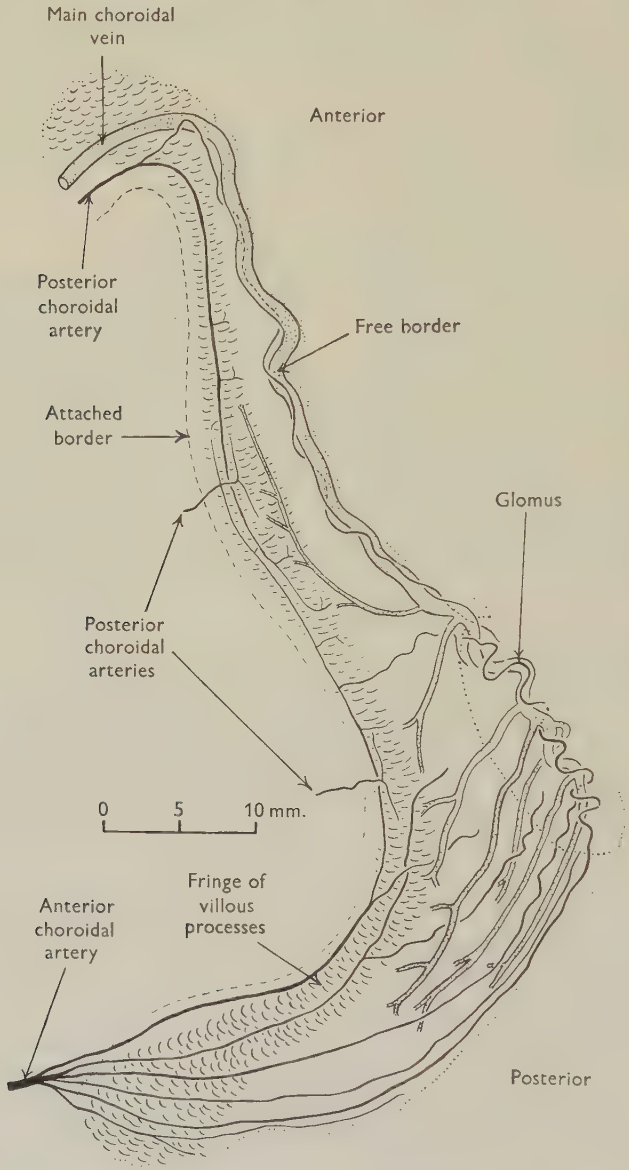
The choroid plexus of the lateral ventricle has the general appearance of a thin undulating membrane (Pl. 1, fig. 5). On macroscopic examination, a well-marked swelling is seen on the free edge of the plexus at the junction of the body and posterior horn of the lateral ventricle. Following Findlay (1899), the term *glomus* has been adopted for this swelling. Both surfaces of the plexus exhibit frond-like villous processes. These processes are mainly aggregated into two fringes, one on the superior and the other on the inferior surface of the plexus, close to the attached border. The fringes form the rounded posterior end of the plexus, and anterior to the glomus where the plexus narrows, the fringes approach the free border and are continuous through the interventricular foramen with the plexus of the third ventricle. The free border of the plexus, except at its posterior end, shows very few villi.

The plexus is supplied by anterior and posterior choroidal arteries. The anterior choroidal artery is constantly given off from the internal carotid artery between the posterior communicating artery and the division of the internal carotid artery into its middle and anterior cerebral branches. The anterior choroidal artery gives off branches to structures in the neighbourhood, and the branch which runs to the plexus crosses the optic tract and enters the choroidal fissure at the anterior end of the inferior horn of the ventricle. Immediately before entering the plexus the artery breaks up into a variable number of branches, usually six to eight.

The posterior choroidal arteries are branches of the posterior cerebral artery. There are usually from three to five branches entering through the choroidal fissure from the tela choroidea. One branch constantly enters the plexus in the region of the interventricular foramen, and anterior branches to the right and left plexuses may arise from a single stem. In some specimens a posterior choroidal branch enters the plexus in the posterior part of the inferior horn of the ventricle.

Free communications between the anterior and posterior choroidal arteries are to be found within the tela choroidea (Pl. 2, fig. 6), in the villous fringe and at the glomus.

Within the plexus both the anterior and posterior arteries give branches which may be broadly divided into branches to the villous fringe and branches to the glomus region. The anterior choroidal branches which enter the hinder end of the plexus are of fairly uniform size. Four or five branches pass towards the free border of the plexus and run parallel to one another towards the glomus (Pl. 2, fig. 7). These vessels give off few side branches and terminate at the glomus. Approaching the glomus they become very tortuous and often appear to form a spiral around the glomus (Pl. 2, fig. 7). Sometimes a branch of the anterior choroidal artery runs directly across the plexus towards the glomus. One or two large branches run forwards in the base of the villous fringe towards the interventricular foramen, and give off branches which enter the fronds (Pl. 2, fig. 8). The anterior branch of the


Main choroidal vein
Anterior
Posterior choroidal artery
Free border
Attached border
Glomus
Posterior choroidal arteries
0
5
10 mm.
Fringe of villous processes
Anterior choroidal artery
Posterior

Text-fig. 2. Diagram to illustrate the principal features of the human choroid plexus. (Lateral ventricle: right side.)

posterior choroidal artery breaks up on entering the plexus. Fine parallel branches, three to four in number, similar to those of the anterior choroidal artery, run posteriorly along the free border, closely related to the large vein in its edge, and end in the region of the glomus. A large branch runs along the base of the villous fringe and shares with the corresponding vessel from the anterior choroidal artery in the supply of the fronds. The posterior branch of the posterior choroidal artery enters the plexus in the inferior horn and runs forwards close to the attached border supplying the villous fronds. It may give off a branch which runs directly to the glomus. The intermediate branches of the posterior choroidal artery join the plexus from the tela choroidea and assist mainly in the supply of the villous fringe.

From the main vessels in the base of the fringe short stems arise which divide to supply the fronds. The appearance is that of a marginal artery giving off short terminal branches along its length rather than that of an arterial tree with branches of ever-diminishing size (Pl. 2, fig. 8).

In general, the anterior choroidal artery supplies the more posterior part of the plexus while the posterior choroidal arteries supply the more anterior part. The variations seen and the communications observed between these vessels suggest, however, that the plexus is indifferently supplied from both anterior and posterior choroidal arteries.

The plexus shows an extremely rich venous network. The venous radicles begin in the villous fronds and unite to form large venules which run towards the glomus (Pl. 2, fig. 9). At the glomus these vessels are intimately related to branches of the choroidal arteries. The veins join to form a single large stem which runs forwards from the glomus in the free edge of the plexus (Pl. 1, fig. 5), accompanied by fine parallel arteries (vide supra). At the interventricular foramen this vein is joined by the thalamostriate vein and a vein from the septum lucidum before turning into the tela choroidea to form the internal cerebral vein. In some specimens a vein leaves the plexus near the posterior end and joins the basal vein. In one specimen a large vein can be seen running from the glomus to join the internal cerebral vein in the tela choroidea.

On entering a frond the arteriole joins a network of capillary loops (Pl. 2, fig. 10), which connect with the venous radicles. It was noted that with injections of carbon particles and the red dispersion, the injection mass passed rapidly over into the capillaries and veins and was not found in the arteries. On the other hand, the blue dispersion medium tended to remain on the arterial side and was seldom to be found in the veins. Arteriovenous connexions without the intervention of a capillary system were also to be observed in some of the fronds (Pl. 2, fig. 11), but it is not possible to state the frequency with which these occur.

DISCUSSION

The surfaces of the choroid plexus of the lateral ventricle present numerous frond-like processes. The distribution of these processes differs in different animals; in the rabbit the villi are most numerous at the anterior end close to the interventricular foramen, whereas in the human plexus the fronds are aggregated mainly as a fringe close to the attached border of the plexus and extending along its whole length. The remainder of the surface shows only scattered single villi, and even these are absent throughout the greater part of its length. Hudson & Smith (1952) have noted that in the sheep the fronds were most numerous at the rostral end of the plexus. It is to be expected, therefore, that, in accordance with the simpler arrangement of the villous processes, the vascular pattern of the plexus in the rabbit will be of a less complex nature than that of the human choroid plexus where there is a great concentration of villous processes along the attached border. Findlay (1899) has noted the shaggy appearance of certain parts of the choroid plexus but has not stated where these parts are to be found.

In all the human plexuses examined the glomus was a well-defined landmark. It presents as an oval-shaped swelling at the junction of the body and posterior horn of the ventricle. In cleared specimens the glomus has a cystic appearance, and hyaline concentric bodies were found to be constantly present in large numbers in the adult plexuses. They were absent in all the foetal plexuses examined and in one specimen from a 6½-year-old boy. No structure resembling the glomus is to be seen in the choroid plexus of the rabbit, although the vascular relationships suggest that the most nearly corresponding region is that near the interventricular foramen where the free border bends medially at almost a right angle. At this place, in the rabbit, the anterior and posterior choroidal arteries meet and the main choroidal vein begins.

In the rabbit (and also in the rat) there is a curious long tongue-like process for which the term *lingula* has been suggested. A similar process is not found in the adult human plexuses, but in some foetal specimens an indication of such a process is to be found posterior to the glomus. It may be that the lingula is buried in the human choroid plexus by the greater folding and growth of this part of the plexus. It is possible that both the glomus and the lingula play a definite part in the control of the production of the cerebrospinal fluid, perhaps as mechanisms sensitive to the pressure of the fluid in the ventricles, but until further investigations have been made it is not profitable to speculate as to how they might perform such a function.

An interesting point which emerges from the examination of the choroid plexus of the rabbit is the junction of the main continuation of the anterior choroidal artery with the posterior choroidal artery at the anterior angle. This is clearly seen in the illustration (Pl. 1, fig. 3). In several specimens the connexion is not to be seen, but the terminations of the two vessels approach within a millimetre of each other. This suggests that in these cases the injection mass advancing simultaneously along both arteries pushed a small quantity of fluid in front of it so that the continuity of the two arteries was not demonstrated. The connexion observed is comparable in degree of magnitude with the connexions between the meningeal vessels illustrated by Pfeifer (1930). Similar connexions were observed in the human choroid plexuses and their significance is discussed later.

In both the rabbit and man, the vessels become increasingly tortuous as the area of villous thickening is reached. A similar condition has been observed in the choroid plexus of the sheep by Hudson & Smith (1952), and an identical appearance has also been noted in the choroid plexus of the goat. It will be observed in the illustration of the choroid plexus of the rabbit that the anterior choroidal artery becomes most tortuous just at its junction with the posterior choroidal artery (Pl. 1, fig. 3). This mechanism may have some importance as a method of reducing pressure, so that there is not too steep a pressure gradient between the blood entering the villous process and the cerebrospinal fluid.

A point which is not stressed in the standard text-books of anatomy is that there are generally four or five separate posterior choroidal arteries coming from the posterior cerebral artery (Beevor, 1907). Even in the rabbit there are generally two or three separate posterior choroidal arteries to be seen.

The venous supply of the plexus in the rabbit is remarkable for the rich network of venules gathering together at the anterior end of the plexus into one large choroidal vein. There is a very scanty network in the posterior part of the plexus with a few small veins accompanying the anterior choroidal artery. A similar picture is seen in the human, and it appears probable that the bulk of the blood enters the plexus through the anterior choroidal artery at the posterior end of the plexus and leaves through the choroidal vein at the anterior end.

The outstanding feature which emerges from an examination of the specimens is the vascular relations of the frond-like villous processes. The villous process is better developed in man than in the rabbit, and it is supplied by an arteriole which is connected to the venous system by both capillary loops and occasional arterio-venous connexions having a diameter of 20–30μ.

The vascular relations of this villous frond have aroused both interest and speculation. Putnam & Ask-Upmark (1934) have noted the presence of what they have termed 'unusually coarse thin-walled capillaries'. These appear to be identical with the large capillaries commented upon by Findlay (1899), who has queried whether a sinusoidal mechanism is present in the plexus but has concluded that such a mechanism does not exist. In the present investigation, the capillary loops observed while showing irregularities appeared to be well within the normal range of capillary size. Putnam & Ask-Upmark (1934) have also remarked that between the large arteries and veins and the coarse capillaries they have described, there are few vessels of intermediate size. The question arises whether these authors, who examined the exposed choroid plexus in the living cat, were able to observe the true capillary bed. It is possible that they may have seen the blood passing from the arterioles to the venules through the arteriovenous connexions, whilst the true capillary bed was closed down. This would readily account for the 'unusually little difference in colour between arterial and venous blood as seen under the microscope' which they have noted in their examination of the plexus.

The main object of this investigation was to ascertain whether the arrangement of the vessels in the choroid plexus suggested any point at which nervous control of the plexus might be éffected, and thus the production of the cerebrospinal fluid regulated. All the evidence points to such control, if present, being exercised at the periphery of the plexus. It appears unlikely that there is any point at which the anterior choroidal artery or its choroidal branch is controlled, so that the blood flow distal to that point is restricted. The least anatomically variable and clinically most important part of the anterior choroidal artery is the distal part, after the choroidal branch is given off, which supplies the lateral geniculate body (Abbie, 1933). There is unlikely to be an arrangement whereby the blood supply to the geniculate body is determined by the requirements of the choroid plexus. Secondly, as has been noted above, the choroidal branch divides immediately before entering the plexus into five or six branches, each almost as large as the parent stem. This reduces the length of the anterior choroidal artery where its circulation may be effectively controlled by a local nervous mechanism to a bare minimum. When the free communications between the anterior and posterior choroidal arteries and the multiplicity of the choroidal branches of the posterior cerebral artery are also considered, it seems impossible that the cerebrospinal fluid production is effectively altered by any nervous mechanism acting on the main stem of the anterior choroidal artery. In our opinion the anatomical evidence tends to show that if a nervous control is present it must be exercised at the periphery.

SUMMARY

1. The vascular patterns of the choroid plexus of the lateral ventricle have been studied in the rabbit and man by the use of injection masses and the examination of whole cleared specimens.

2. The choroidal villi are not uniformly distributed over the surface of the plexus. Scattered single villi are present over the greater part of both surfaces, but the main aggregations are found, in the rabbit, at the anterior end of the plexus, and, in man, along a fringe close to the attached border and extending the whole length of the plexus.

3. The choroid plexus of the lateral ventricle is supplied by a single branch from the anterior choroidal artery, itself a branch of the internal carotid artery, and by several posterior choroidal arteries arising independently from the posterior cerebral artery. These arteries communicate within the plexus and in the tela choroidea.

4. There is a rich venous plexus whose radicles unite in the region of the glomus to form a single large vein which leaves the plexus at the interventricular foramen to join the internal cerebral vein.

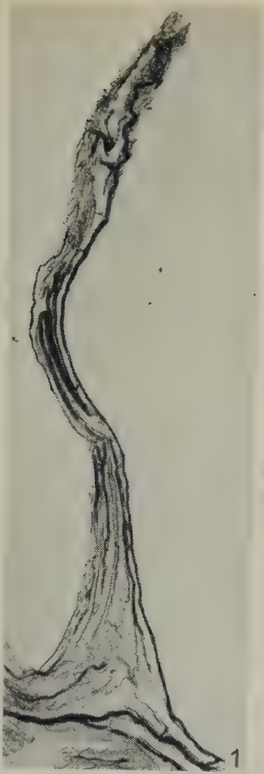
5. Within the choroid plexus in man the branches of the choroidal arteries are of two main varieties—(1) fine parallel vessels with few side branches which run along the free border of the plexus to end in the region of the glomus, and (2) branches which run along the base of the villous fringes close to the attached border of the plexus, giving off short stems to supply the fronds.

6. Each frond is supplied by an arteriole which breaks up into capillary loops which are connected within the villous process to the terminal venule. Arteriovenous connexions having a diameter of 20–30μ also occur within the fronds.

7. A glomus is constantly present in all the human plexuses examined, but cannot be identified in the choroid plexus of the rabbit. In the rabbit, a curious tongue-like process, the lingula, projects from the free border of the plexus behind the 'glomus' region.

8. The significance of these findings in the interpretation of possible mechanisms for the control of the production of the cerebrospinal fluid is discussed.

We are greatly indebted to Prof. J. D. Boyd for his constant interest, advice and encouragement. Our thanks are also due to Mr J. A. Fairfax Fozzard for the photographic work.



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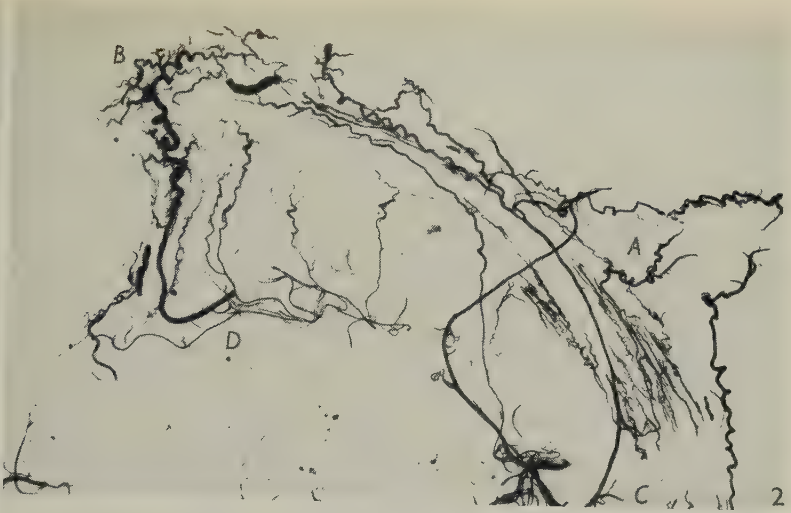

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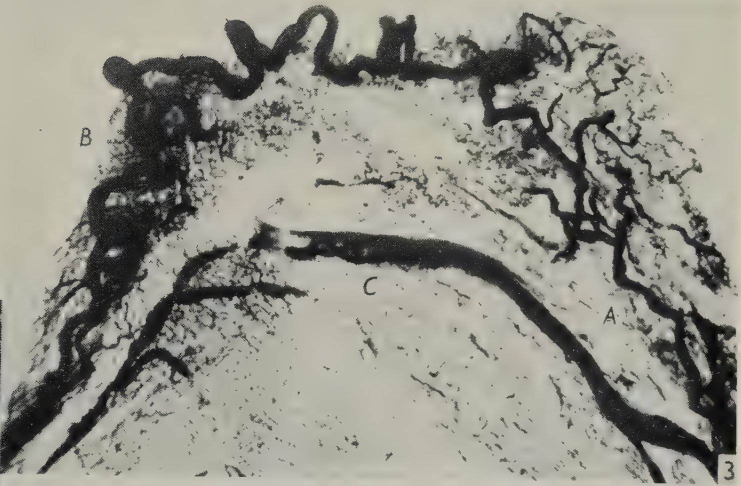

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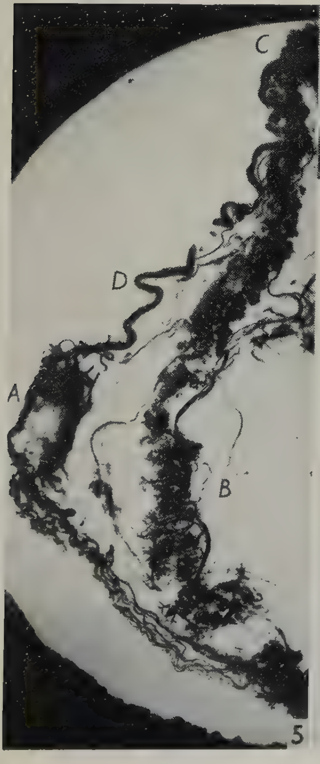

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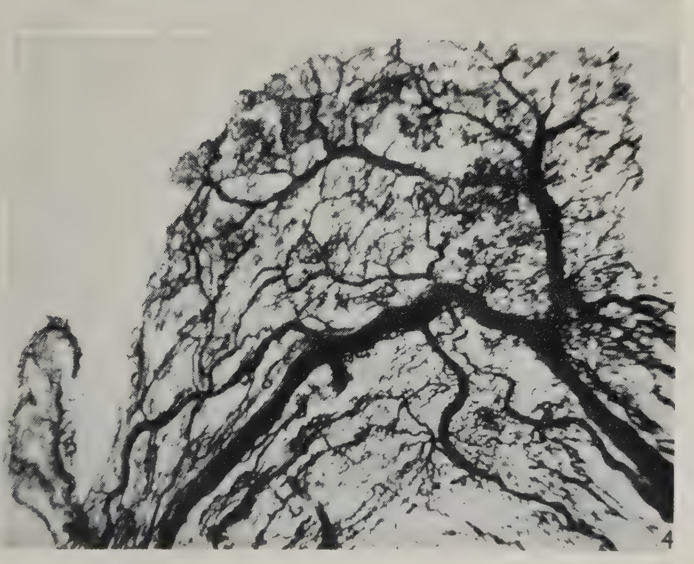

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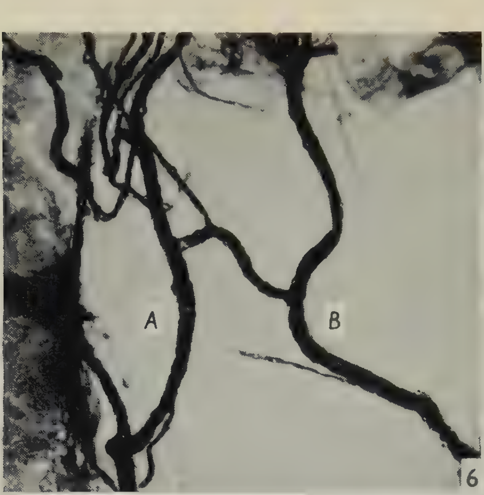
MILLEN AND WOOLLAM—VASCULAR PATTERNS IN THE CHOROID PLEXUS



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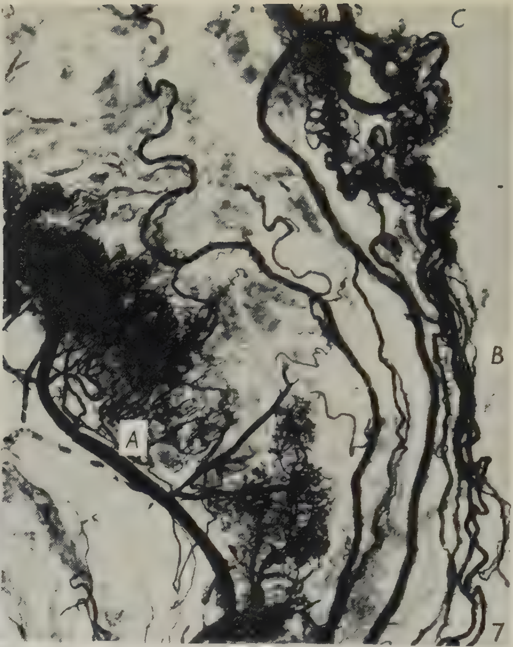

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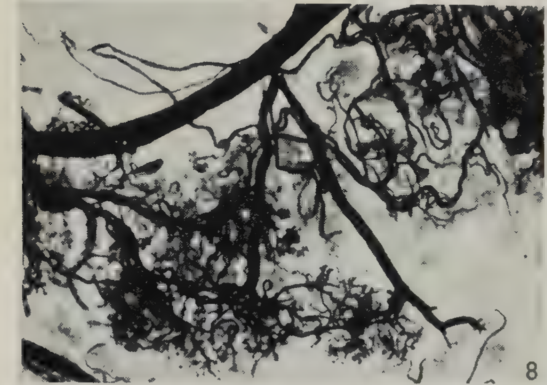

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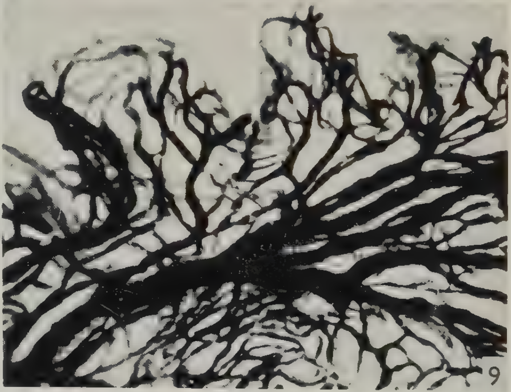

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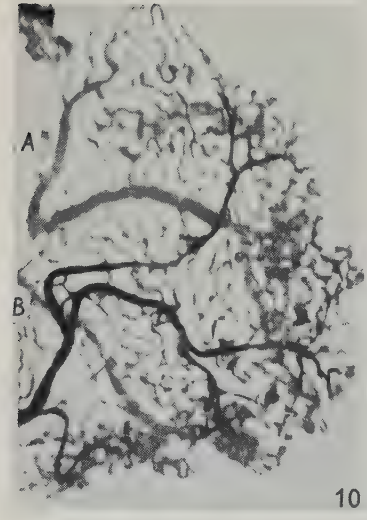

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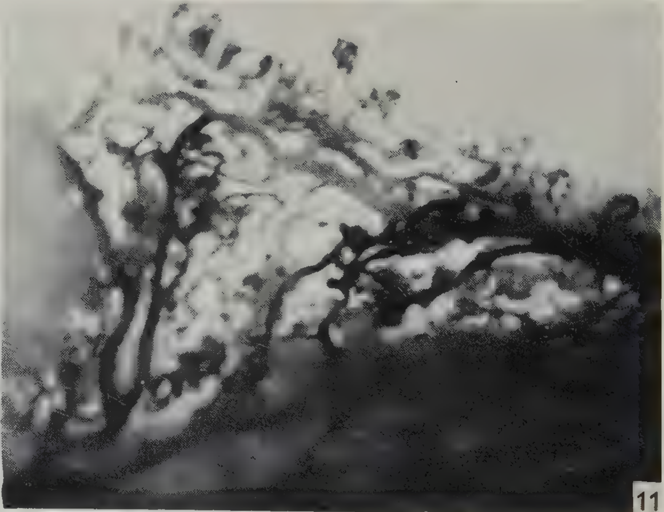

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MILLEN AND WOOLLAM—VASCULAR PATTERNS IN THE CHOROID PLEXUS

REFERENCES

Abbie, A. A. (1933). The clinical significance of the anterior choroidal artery. *Brain*, **56**, 233–246.

Beevor, C. E. (1907). The cerebral arterial supply. *Brain*, **30**, 403–425.

Dandy, W. E. (1919). Experimental hydrocephalus. *Ann. Surg.* **70**, 129–142.

Findlay, J. W. (1899). The choroid plexuses of the lateral ventricles of the brain, their histology, normal and pathological (in relation specially to insanity). *Brain*, **22**, 161–202.

Flexner, L. B. (1933). Some problems of the origin, circulation and absorption of the cerebrospinal fluid. *Quart. Rev. Biol.* **8**, 397–422.

Hudson, A. J. & Smith, C. G. (1952). The vascular pattern of the choroid plexus of the lateral ventricle. Paper read at the American Association of Anatomists Annual Session. Summarized in *Anat. Rec.* **112**, 345–346.

Pfeifer, R. A. (1930). *Grundlegende Untersuchungen für die Angioarchitektonik des menschlichen Gehirns.* Berlin: Julius Springer.

Putnam, T. J. & Ask-Upmark, E. (1934). The cerebral circulation. XXIX. Microscopic observations on the living choroid plexus and ependyma of the cat. *Arch. Neurol. Psychiat., Chicago*, **32**, 72–80.

Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J. & Prichard, Marjorie M. L. (1947). *Studies of the Renal Circulation.* Oxford: Blackwell.

Weed, L. H. (1922). The cerebrospinal fluid. *Phys. Rev.* **2**, 171–203.

EXPLANATION OF PLATES

The photographs are all of whole cleared preparations. Figs. 1–4 are of the choroid plexus of the rabbit, fig. 5–11 are of human choroid plexuses.

Plate 1

Fig. 1. Left choroid plexus showing lingula. Injected with red and blue dispersions. ×8.

Fig. 2. Arteries of right choroid plexus. Injected with blue dispersion. ×7. *A*, base of lingula; *B*, anterior angle; *C*, branches of anterior choroidal artery; *D*, posterior choroidal artery.

Fig. 3. Right choroid plexus showing the junction of the anterior and posterior choroidal arteries at the anterior angle. Injected with red and blue dispersions. ×22·5. *A*, anterior choroidal artery; *B*, posterior choroidal artery; *C*, choroidal vein.

Fig. 4. Left choroid plexus showing the choroidal vein and some of its tributaries. Injected with red dispersion. ×22·5.

Fig. 5. Left choroid plexus showing villous fringe and glomus. Injected with blue dispersion. ×1·3. *A*, glomus; *B*, villous fringe, *C*, site of interventricular foramen; *D*, main choroidal vein.

Plate 2

Fig. 6. Left choroid plexus showing communication between the anterior and posterior choroidal arteries in the tela choroidea. Injected with blue dispersion. ×5. *A*, anterior choroidal artery; *B*, posterior choroidal artery. ×5.

Fig. 7. Right choroid plexus showing the two main types of branches of the anterior choroidal artery. Injected with blue dispersion. ×4. *A*, branch of anterior choroidal artery in base of villous fringe; *B*, parallel arteries in free border; *C*, glomus.

Fig. 8. A section of the same plexus as fig. 7 at a higher magnification showing short side branches of choroidal arteries supplying fronds of the villous fringe. Injected with blue dispersion. ×8.

Fig. 9. Right choroid plexus of 6 months' foetus showing venous pattern. Injected with Indian ink and plasma. ×22·5.

Fig. 10. A villous frond showing the capillary network. Injected with red and blue dispersions. ×30. *A*, venule; *B*, arteriole.

Fig. 11. Villous frond showing an arteriovenous connexion. Injected with red and blue dispersions. ×52.

A PRESOMITE HUMAN EMBRYO SHOWING AN EARLY STAGE OF THE PRIMITIVE STREAK

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Although many human embryos of a presumed age of 15–17 days showing early stages of the primitive streak have been reported in the literature, most of them show specific characteristics (compare, for example, Möllendorff (1925) and Florian & Hill (1935)), while others have suffered distortion during fixation and preparation for histological examination (Bryce, 1925). When an embryo of this age group in a moderately well-preserved state was discovered by one of us (T.N.A.J.), it was decided to describe it as a contribution to the literature on such embryos. We propose to designate the specimen 'Liverpool I'.

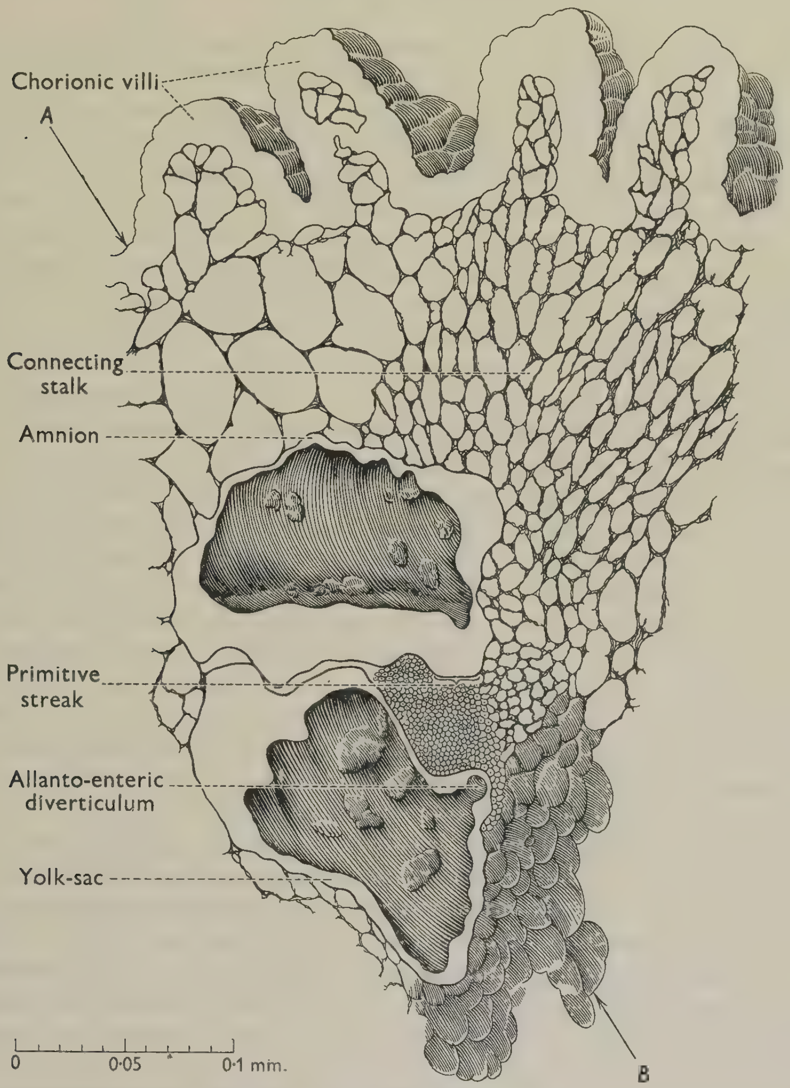
CLINICAL HISTORY OF THE EMBRYO

The embryo was obtained from a woman aged 37 years, married 12 years, who has four children, the youngest being 2 years of age. She has also had two abortions, the last one occurring in 1942. In June 1951, she sought medical advice on account of epimenorrhagia, her menstrual cycle for the previous few months having consisted of rather heavy bleeding lasting 7–8 days and commencing every 21–28 days.

Uterine curettage was performed on 21 June 1951, and the embryo found during routine histological examination of the curettings. The last menstrual flow before operation commenced on 24 May 1951 and was more normal than previously, lasting only 5 days; coitus occurred on 1, 5 and 13 (or 14) June. From the histological features of the embryo, it appears most likely that conception occurred on 5 June. Curettage was carried out on the day when menstruation might have been expected to begin, and the evidence suggests that conception took place 16 days previously, that is, on the twelfth day of the cycle. The curettings were fixed in Bouin's fluid, and one edge of the blastocyst was discovered in the first section cut and examined. Fortunately, the embryo itself was intact, and 7 μ sections of the remainder of the paraffin block were taken and stained with Ehrlich's haematoxylin and eosin. The embryo is found in twenty-three sections (section numbers 24–46), the sections being transverse to the antero-posterior axis of the embryo at an angle of 32° from the vertical.

THE BLASTOCYST

It is only possible to measure the dimensions of the chorion in two planes, the external dimensions (including trophoblastic villi) being $1{\cdot}86 \times 1{\cdot}47$ mm., and the internal dimensions of the cavity $1{\cdot}50 \times 0{\cdot}84$ mm. The blastocyst forms an elevation on the decidua, and a closing plug consisting of fibrin whose maximum diameter is 1·05 mm. is present. The villi consist almost entirely of cytotrophoblast, with very little syncytiotrophoblast, and well-marked cores of chorionic (extraembryonic) mesoderm; they are only beginning to show evidence of branching (Pl. 1, fig. 1), and are more well-developed at the embryonic pole of the blastocyst. Lacunae have formed and the syncytiotrophoblast is most clearly seen in relation to their walls. The extraembryonic mesoderm lines the trophoblast and forms a connecting stalk at the caudal end of the embryo (Text-fig. 1) where it is clearly continuous with cells being formed from the primitive streak. Mitotic activity is present in all tissues of the chorion.



Text-fig. 1. A drawing of a reconstruction model of the embryo. Note the slight unevenness of the inner surface of both amniotic cavity and yolk-sac. The line *AB* on the figure indicates the plane of sectioning the embryo.

THE EMBRYO

Yolk-sac and amniotic cavity are present and the embryonic disc is bilaminar, except at the caudal end of the embryo where cells produced from the primitive streak are interposed between ectoderm and endoderm. A reconstruction model of the embryo was made at a magnification of 680 × from $\frac{3}{16}$ in. thick Perspex. By direct measurement, and by estimation from the reconstruction model, the size of the embryonic disc is found to be 0·161 × 0·199 × 0·033 mm., the disc being wider in its transverse diameter. The volume of the amniotic cavity is slightly larger than that of the yolk-sac, and a small allanto-enteric diverticulum, 0·021 mm. in length, is present.

At the anterior end of the embryonic plate one unusual feature is the presence of a thickening of the endoderm, bulging into the cavity of the yolk-sac (Text-fig. 1, and Pl. 1, fig. 2); this appearance, for which it is difficult to provide an explanation, is seen in four sections. Owing to the oblique sectioning of the embryo, the amniotic cavity is not seen until the sixth section (Pl. 1, fig. 3). In this section and the next succeeding one, extraembryonic mesoderm cells are arranged over the apex of the amniotic sac to simulate an amniotic duct which, however, has no communication with the amniotic cavity itself. The endodermal cells of the embryonic disc at this level are arranged quite irregularly, in sharp contrast to the ectodermal cells which have a clearly marked basement membrane. More posteriorly, at the ninth section, the width of the endoderm in the embryonic disc is clearly less than that of the ectoderm (Pl. 2, fig. 1); the transition from the tall columnar cells of disc ectoderm to the flattened cells of amniotic ectoderm is quite abrupt at this level, and the extraembryonic mesoderm is beginning to increase in quantity around the yolk-sac from the single layer of cells seen more anteriorly. In the eleventh section (Pl. 2, fig. 2) there are six layers of extraembryonic mesoderm cells in places at the lateral margins of the yolk-sac, which itself is smaller in size. In the succeeding section (Pl. 2, fig. 3) the yolk-sac can be seen to have narrowed considerably from side to side, so that the area of contact of endoderm with ectoderm is reduced to about one-fifth of the area of the latter. The yolk-sac proper is now restricted to the upper part of the endodermal cavity; the lower part of this cavity is continuous with the allanto-enteric diverticulum (Pl. 2, fig. 4) which continues through the next three sections embedded in the connecting stalk mesoderm.

By the sixteenth section no evidence of endodermal tissue is to be found, but interesting changes can be observed in the ectoderm of the embryonic disc, which are even more pronounced in the following section (Pl. 2, fig. 5); the basement membrane is indistinct, particularly in the axial region of the disc, and cells are passing from the ectoderm into the mesodermal tissue of the connecting stalk. There is also evidence of considerable cellular activity in this region, as shown by the more irregular arrangement of cells and occasional mitotic figures. This appearance is also seen in the eighteenth section (Pl. 2, fig. 6), and extends therefore over three sections (0·021 mm.). These histological features are taken to represent an early stage of the primitive streak.

The remainder of the sections show clearly the major part of the connecting stalk between embryo and chorion, and the gradual diminution in size of the amniotic cavity.

DISCUSSION

Of the already reported embryos of a similar age and stage of development, this specimen most resembles the embryo of Schlagenhaufer & Verocay (1916), the OP (Möllendorff, 1921) and WO (Möllendorff, 1925) embryos, the Bi I (Florian, 1927) embryo and Edwards-Jones-Brewer ovum (Brewer, 1938). All of these specimens have measurements very similar to those of our embryo, and have many other features in common. The embryo of Schlagenhaufer & Verocay (1916), for example, possesses an allanto-enteric diverticulum but no amniotic duct; there is no primitive streak, however. The primitive streak in Möllendorff's (1921) OP embryo is longer (50 μ), and the allanto-enteric diverticulum more well-developed, while Möllendorff's (1925) WO embryo is younger in having a solid allanto-enteric diverticulum and no primitive streak, although the measurements of the embryonic disc in the WO embryo are slightly larger than in our specimen. Florian's (1927) Bi I embryo exhibits similar histological features, but is slightly larger in size, and possesses an amniotic duct. The Edwards-Jones-Brewer ovum has a primitive streak which is larger (40 μ), and no allanto-enteric diverticulum, but the embryonic disc is almost the same size as in our specimen, and the endodermal cells similarly do not extend to the margins of the disc. The histological features of our specimen also resemble those of the Carnegie 7801 embryo (Heuser, Rock & Hertig, 1945), except for the greater degree of histological development of the primitive streak in the latter.

The primitive streak in this embryo occupies an area at the extreme posterior end of the ectoderm of the embryonic plate (Text-fig. 1). Immediately ventral to this is a collection of cells which shows characteristics not previously reported in other embryos of a similar age, and which may therefore indicate some abnormality. Although the allanto-enteric diverticulum is enclosed by these cells, which must consequently be regarded as belonging to the connecting stalk, at least in part, the transition between them and the tissue of the connecting stalk proper at the posterior end of the embryo is abrupt (compare, for example, the disposition of these cells in Pl. 2, fig. 5 and the disposition of cells in the connecting stalk proper in Pl. 2, fig. 6), since the cells in the latter structure are much less compactly arranged. This suggests that the origin of the mass of cells ventral to the primitive streak is not from extraembryonic mesoderm, but from primitive streak, since cells are actively proliferating from the ventral aspect of this structure. The unusual feature of this mass of cells, however, is that it extends to a level anterior to the primitive streak, ventral to a part of the ectoderm which has an intact basement membrane (Pl. 2, fig. 4). The cells in this region are presumably mainly of primitive streak origin, although they are continuous still further anteriorly with extra-embryonic mesoderm cells at the lateral margins of the yolk-sac (Pl. 2, figs. 2 and 3). If this embryo is normal, therefore, the condition represented may be an early stage of the primitive streak, which has differentiated in a small area at the caudal end of the embryonic plate, and is proliferating cells anteriorly, ventral to a part of the ectoderm with an intact basement membrane which will also eventually form the definitive primitive streak, before this structure is able to grow in length by the addition of new ectodermal cells at its caudal end.

There are signs in this specimen that the yolk-sac may not be quite normal. Although the shape of this structure does not exactly conform to that in the Carnegie 7801 embryo, it is very similar to the yolk-sac in the Bi I embryo. There is also some evidence in the more anterior sections (as shown in Pl. 1, fig. 3) of a shrinkage of endodermal cells of the embryonic plate, so that they are becoming detached from overlying ectoderm.

The characteristics of this specimen are therefore quite specific as regards size and degree of development. Its resemblance, in many features, to previously reported embryos, confirms the age of 16 days estimated from the clinical history.

SUMMARY

A presomite human embryo of an estimated age of 16 days has been recovered from uterine curettings. It shows an early stage of the primitive streak, which is 21 μ in length. The embryo also has many features previously reported in embryos of a similar age.

This work was aided by a grant from the Medical Research Council. We wish to thank Mr L. G. Cooper for his technical assistance.

REFERENCES

Brewer, J. I. (1938). A human embryo in the bilaminar blastodisc stage (The Edwards-Jones-Brewer Ovum.) *Contr. Embryol. Carneg. Instn*, **27**, 85–94.

Bryce, T. H. (1925). Observations on the early development of the human embryo. *Trans. Roy. Soc. Edinb.* **53**, 533–567.

Florian, J. (1927). Über zwei junge menschliche Embryonen. *Anat. Anz.* Erg. **63**, 184–192.

Florian, J. & Hill, J. P. (1935). An early human embryo (No. 1285, Manchester Collection), with capsular attachment of the connecting stalk. *J. Anat., Lond.*, **69**, 399–411.

Heuser, C. H., Rock, J. & Hertig, A. T. (1945). Two human embryos showing early stages of the definitive yolk sac. *Contr. Embryol. Carneg. Instn*, **31**, 85–99.

Möllendorff, W. von (1921). Über einem jungen, operativ gewonnen menschlichen Keim (Ei OP). *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **62**, 406–432.

Möllendorff, W. von (1925). Das menschliche Ei WO(lfring). Implantation, Verschluss der Implantationsöffnung und Keimesentwicklung beim Menschen vor Bildung des Primitivstreifens. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **76**, 16–42.

Schlagenhaufer, F. & Verocay, F. (1916). Ein junges menschliches Ei. *Arch. Gynaek.* **105**, 151–168.

EXPLANATION OF PLATES

Plate 1

Fig. 1. A low-power photomicrograph of the blastocyst (section 35). The chorionic villi are only beginning to divide, and the lacunae are well developed. The closing plug is clearly shown in the lower part of the figure. (×65.)

Fig. 2. A section (number 27) of the anterior end of the embryo, showing the thickening of the endoderm (at *a* in the figure) bulging into the cavity of the yolk-sac. Since the embryo was cut obliquely, only the disc ectoderm and not the amniotic cavity is seen in this section. Part of the wall of the yolk-sac was torn during histological sectioning (just below the pointer in the figure), and was displaced into the blastocyst cavity. (×180.)

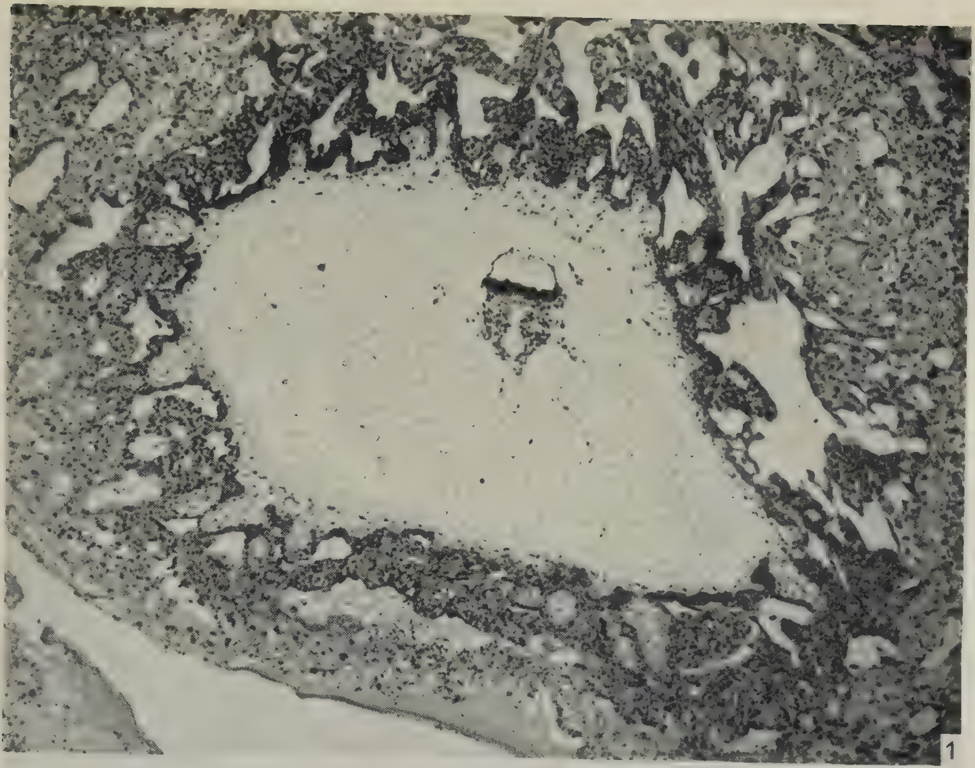
Fig. 3. Photomicrograph of section 29, showing the amniotic cavity and the arrangement of extraembryonic mesoderm cells over its apex to simulate an amniotic duct (*s.a.d.*) which does not communicate with the amniotic cavity. (×180.)

Plate 2

(All figures are at a magnification of ×180.)

Fig. 1. The embryo at a more posterior level (section 32) showing the diminution in width of disc endoderm. In this, and the next four succeeding figures, the transition from the tall columnar cells of disc ectoderm to the flattened amniotic ectodermal cells is clearly marked.

Fig. 2. Photomicrograph of section 34 showing the increased number of extraembryonic mesoderm cells at the lateral margins of the yolk-sac. The ectodermal cells in the centre of the disc are slightly irregular, simulating the formation of neural folds.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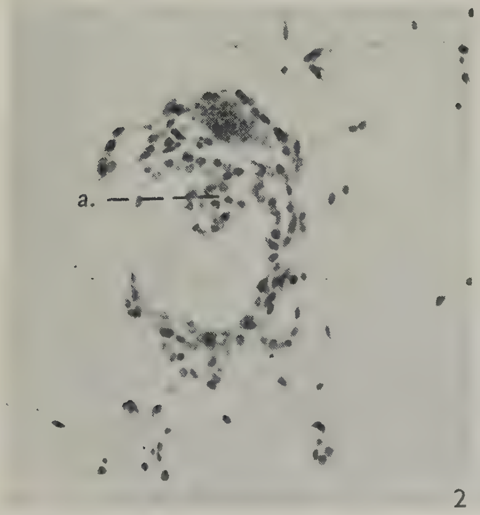

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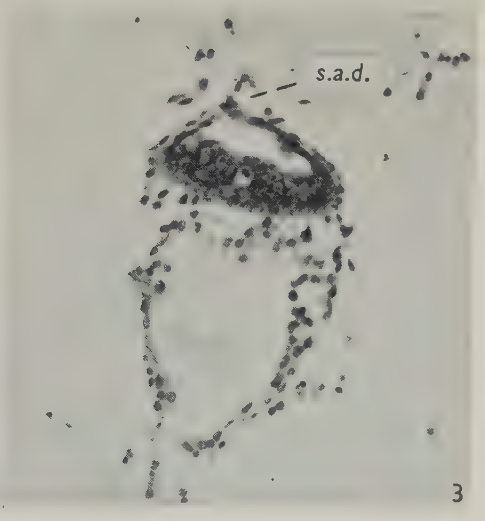

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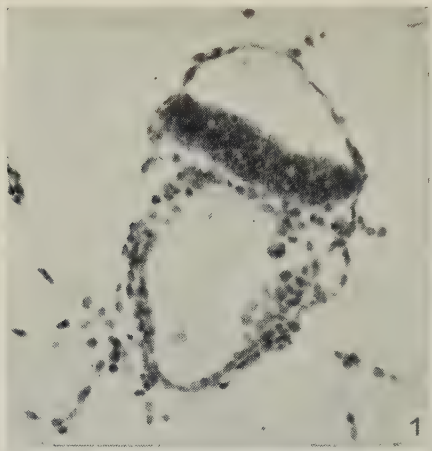
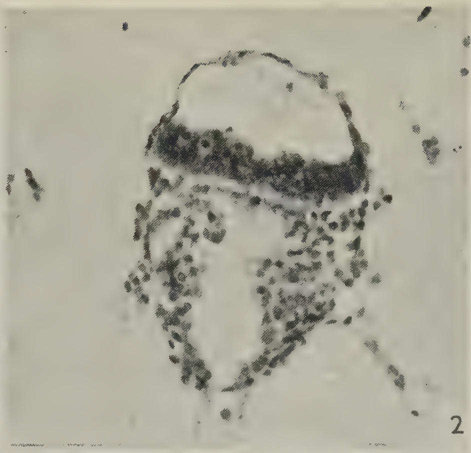
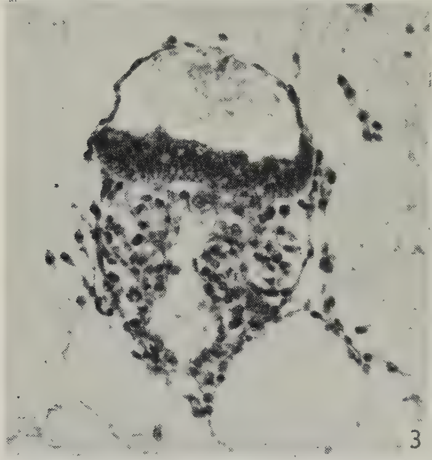
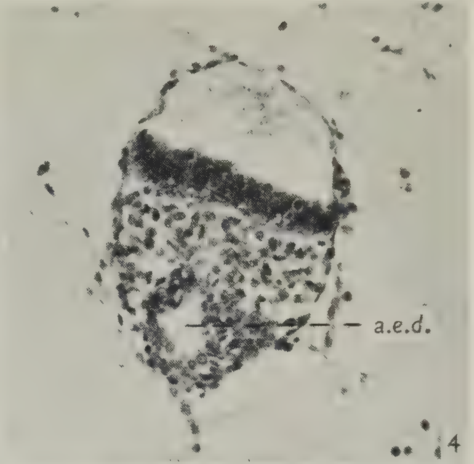
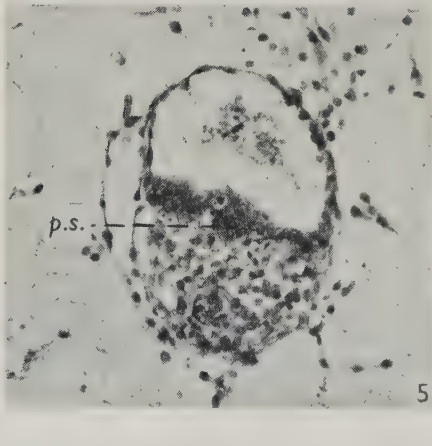
HARRISON AND JEFFCOATE—PRESOMITE HUMAN EMBRYO

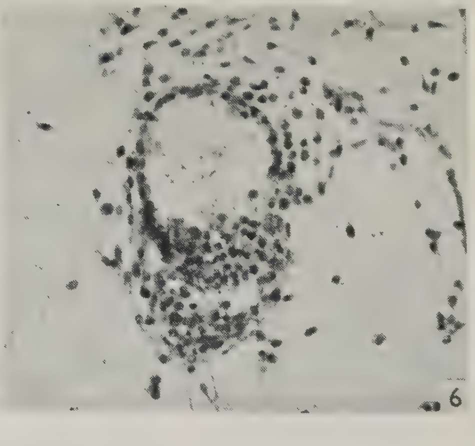












HARRISON AND JEFFCOATE—PRESOMITE HUMAN EMBRYO

Fig. 3. The narrowing of the yolk-sac in its transverse diameter as seen in section 35. There is also an indication of the division of the yolk-sac into yolk-sac proper, and the allanto-enteric diverticulum (in the lower part of the endodermal cavity).

Fig. 4. Photomicrograph of section 36 to show the allanto-enteric diverticulum (*a.e.d.* in the figure) embedded in connecting stalk mesoderm.

Fig. 5. The region of the embryo (section 40) in which the disc ectoderm cells are clearly dividing (the primitive streak; *p.s.* in figure) to contribute cells to the connecting stalk. A mitotic figure can be seen in this region.

Fig. 6. Section 42 of the embryo at the posterior end of the primitive streak in which there is no clear distinction between ectodermal cells and cells of the connecting stalk. No endodermal tissue is present at this level, and only the amniotic cavity can be seen. The part of the connecting stalk between embryo and chorion is seen in the top right-hand corner of the figure.

STUDIES ON THE MORPHOGENESIS OF THE MOUSE STERNUM

III. EXPERIMENTS ON THE CLOSURE AND SEGMENTATION OF THE STERNAL BANDS

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INTRODUCTION

The study of the normal embryonic development of the mouse sternum and the experiments on its differentiation *in vitro* reported in Parts I and II of the present series of studies (Chen, 1952*a*, *b*) indicated two points of special interest in relation to the developmental mechanics of this bone, namely the closure of the two sternal bands and the segmentation of the sternum.

The bilateral, dual origin of the sternum is perhaps an unusual feature in morphogenesis, for very few organs in the vertebrate have a similar ontogenetic course, but in all classes the true sternum originates in the same way (Gladstone & Wakeley, 1932). So far, the mechanism by which the two widely separated mesodermal bands are brought together has only been investigated experimentally in birds (Fell, 1939). Fell has shown that, of the factors possibly involved, i.e. (i) the expansion of the sternal bands themselves, (ii) the elongation of the ribs, (iii) the shrinkage of the mid-ventral body-wall and (iv) the movement of the dorso-lateral tissue of the thoracic wall, the last one is primarily responsible, while the shrinkage of the ventral body-wall may have a secondary effect.

The study of the normal development of the mouse sternum has shown that all these factors except the first may operate in the mouse also. By analogy, it seemed possible that the same mechanism was involved in both species, but it was advisable to investigate the matter by experimental means. Section A accordingly is devoted to investigations made to find (*a*) whether the closure of the bands is due to the elongation of the ribs, (*b*) whether there is a movement of the undifferentiated tissue in the ventral body-wall in a median direction, (*c*) if so, whether it is due to the shrinkage of the ventral body-wall or to an active migration, and (*d*) whether the sternal bands can be displaced by this tissue movement.

The problem of the segmentation of the sternum pertains only to the mammals whose sternum ossifies as a series of bony segments separated by bands of cartilage. As briefly mentioned in the discussion on the normal development of the mouse sternum (Chen, 1952*a*), this segmentation seemed secondary in nature and due to the influence of the ribs. Some workers (viz. Bryson, 1945) believe that it is a result of the inhibition by the rib tips of hypertrophy of the cartilage cells at the sternocostal junctions. However, no definite proof has been presented and arguments both for and against this theory are available. The intimate contact of the ribs with the sternal tissue during development, and the fact that the sternal ends of the ribs do not ossify, support the possibility that the rib tissue might exert a mechanical or a chemical effect on the sternal cartilage cells. On the other hand, in birds and certain species of reptiles the attachment of ribs does not result in the segmentation of the sternum (Gladstone & Wakeley, 1932). It seemed obvious that the problem could not be solved by morphological studies alone and an experimental approach was therefore made, the results of which are reported in section B.

A. THE CLOSURE OF THE STERNAL BANDS

TECHNIQUE

To see whether the closure of the sternal bands was due to the elongation of the ribs, the same technique was used as that for the study of the effect of ribs on segmentation which is fully described in section B (p. 136). Briefly, explants were made of embryonic sterna of mice at stage 3 (Chen, 1952*a*) when the bands had not yet fused. The sternal bands were dissected free of all ribs, cultivated by watch-glass method in a medium composed of equal parts of plasma and embryo extract and compared with control explants with ribs attached.

To study the movement of tissues in the ventral body-wall, embryos of slightly younger age (stage 2) were used. The explants were cultivated similarly by watch-glass method. Since the purpose of the experiment was to measure and compare the movement of the structures and of vital marks, subcultivation which usually causes disturbances and contraction of the explants was not practicable. All observations were therefore made on the undisturbed culture during the first 2 or 3 days of growth.

Drawings of the explants were made with the aid of a camera lucida at various intervals. Photographs were also taken of some of the cultures.

EXPERIMENTS AND RESULTS

(1) The elongation of the ribs

As a full account on the effect of the removal of the ribs on the development of the sternum *in vitro* is given in section B (p. 136), it suffices to say here that in the complete absence of the ribs, the two sternal bands still moved medially toward each other; after 24 hr. they were very close together and by the end of the third day had completely fused. As compared with sternal bands with ribs attached, the rate of movement seemed to be slightly faster in the explants without ribs.

Conclusion

The elongation of the ribs is not essential for the closure of the sternal bands.

(2) Tissue movement in the ventral body-wall

Experiments were designed to find whether the undifferentiated tissue in the ventral body-wall can move towards the mid-line in the absence of all or various amounts of the lateral differentiated tissues.

Material and methods

The ventral body-wall of 13-day embryos were excised with very little of the differentiated tissues, i.e. pectoral muscles, sternal bands and clavicles, attached to the edges of the explants. In a few explants, rather more of this differentiated tissue was deliberately left. Since it is difficult to remove the skin at this stage without damaging the tissue underneath, the explants were sometimes cultivated with the ectoderm intact.

To detect tissue movement, the method of using gas black as reference marks was employed. When the carbon particles were first deposited, a few floated in the medium, but most of them were quickly incorporated in the tissue. Gross tissue movement could thus be readily followed by observing the behaviour of these black marks.

To make sure that nutrients from the medium to the tissue were not seriously blocked by the keratinization of the ectoderm, some explants were planted with the skin surface uppermost and others with the skin surface downwards. As will be described below, no essential difference could be detected between the two with regard to the movement of the carbon marks.

For controls, the lateral body-wall of the costal region was cultivated, as normally there is no converging median movement in this region. The skin surface was placed uppermost in all seven cultures.

In both the experimental and control groups, four patches of sterile gas black were laid on each explant. They were arranged in two pairs one behind the other just within the margin (Pl. 1, figs. 1*a*, 1*d*).

All the explants were fixed in acetic Zenker's solution after 36–48 hr. of cultivation and were sectioned. The sections were stained with haematoxylin and chromotrope. Photographs or camera lucida drawings were made of each culture after the explants had lain on the clot for 2 hr. Owing to the initial contraction of the explants *in vitro*, these were found to serve more accurately than the fresh tissue as the initial standard for comparison with subsequent drawings. Other drawings were made on the first and second day of cultivation.

Results

Successive drawings showed that although there was active outgrowth of cells in the explants of the ventral body-wall, the carbon marks moved towards the mid-line (Pl. 1, figs. 1*a*, 1*b*), provided enough dorso-lateral tissue had been included. In the controls, on the other hand, the carbon marks remained stationary or even moved apart (Pl. 1, figs. 1*d*, 1*e*).

(*a*) *Observations on the living cultures.* The typical changes observed in thirty-three cultures during the 2 days' growth may be summarized as follows:

During the first few hours, the explants in both groups contracted a little as usual. The outgrowth of marginal cells migrating radially into the surrounding medium, began after about 10 hr. and continued throughout the period of cultivation. The outline of the original explant, however, could still be readily detected, since the outgrowing marginal tissue was much thinner and less opaque.

Apart from this initial contraction and the migration of the marginal tissues, the two groups of explants behaved quite differently. In the ventral body-wall the carbon marks moved medially so that by the end of the second day of cultivation, the anterior pair of black patches had either fused into one or lay very close together. The degree of their approximation varied a little according to a slight difference in the ages of the embryos from which the explants were taken. Movement was usually faster in the older rudiments and the patches of gas black fused more often. In all the cultures of this series, however, both pairs of carbon marks moved a considerable distance from the margin of the explant toward the mid-line.

This active movement of the tissue in a median direction was further evidenced by the change in size and shape of the explants. In spite of the active migration of marginal cells into the medium which should have caused an increase in area, at the end of the second day *in vitro*, the explants of ventral body-wall remained almost the same size as they were when first explanted. Upon careful examination of the cultures this was seen to be due to an actual shrinkage of the original tissue fragment which, as described above, could be differentiated from the thinner and less opaque outgrowing tissue (not shown in the photographs). Thus after 2 days' cultivation, the outline of the original fragment included an area only half that which it occupied at the time of explantation, while its shape had changed from trapezoid to oblong (cf. Pl. 1, figs. 1*a*, 1*b*). In other words, the shrinkage of the central portion of the explant had counterbalanced the outward migration of marginal cells so that there was no increase in total area.

All these changes seemed the more remarkable and significant when compared with the behaviour of the control explants. In the latter, the carbon marks remained stationary in six explants and moved apart in one. There was practically no change in the general shape of the tissue fragments, and their area had increased as a result of the outgrowth of peripheral tissue. There was no evidence of any internal tissue movement (cf. Pl. 1, figs. 1*d*, 1*e*).

(*b*) *Histological structure*. Serial sections of the explants of the ventral body-wall (Pl. 1, fig. 1*c*) showed them to consist of several layers. First the ectoderm, below which was a thick layer of reticular connective tissue, then the sheet of flattened cells and finally a thin layer of tissue in contact with the clot of the medium.

Normally the sheet of flattened cells in the ventral body-wall of the mouse embryo is double and consists of an inner layer connecting the ventral edges of the sternal bands and an outer layer which is less compact and connects the two masses of pectoral muscle. This double structure was usually more conspicuous in the cultures than in the normal body-wall. In the anterior portion of the explant, the distinction between the two layers was less obvious than further back, where the outer layer was thicker and more uniform than the inner one which contained the degenerate cells mentioned previously (see Chen 1952*a*) and was interrupted by blood vessels. Although the number of degenerate cells, the thickness of the layers, etc., might vary, the area occupied by the sheet of flattened cells was always restricted, being bounded externally by the sternal rudiments and tags of pectoral muscle. The median ectoderm became folded as in normal development but the superficial connective tissue just below the ectoderm had spread laterally far beyond the margins of the flattened cells. This explains the observation on living cultures of the simultaneous outgrowth of tissue from the margins and shrinkage of the original tissue fragment, as shown by the movement of the carbon patches. Evidently the outgrowth was due solely to the peripheral expansion of the superficial tissue, whilst the shrinkage of the original explant was caused by the inward movement of the sheet of flattened cells.

In sections where the carbon particles could be seen (Pl. 1, fig. 1*c*) they were found to be incorporated not only in the ectoderm, on which they were first laid, but also in the connective tissue and occasionally in the sheet of flattened cells, so that their movement could safely be taken as evidence of the movement of these tissues. There was no doubt that such movement involved all three layers, viz. the ectoderm, the connective tissue and the sheet of flattened cells, since the carbon mark moved as a single tidy unit instead of as three units or a diffuse patch which would have resulted had the three layers not moved in unison.

That the superficial tissue, i.e. ectoderm and connective tissue, was carried along by the underlying sheet of flattened cells and did not move in a median direction of its own accord, was suggested by the behaviour of that part of it which was not directly above the layer of flattened cells; thus beyond the outer margins of the latter, the superficial layer spread outwards over the medium like an ordinary tissue culture. It would be difficult to explain why the same layer of tissue should move in two opposite directions unless it were under the influence of other forces. In a culture such as that described above, evidently two forces were in action; one, which may be termed the force of 'unorganized growth' *in vitro*, was the tendency for the peripheral cells to migrate outwards into the medium, whilst the other was the force of active tissue movement toward the median line, an intrinsic activity normal to the sheet of flattened cells between the sternal rudiments of the two sides.

Since these two forces acted in opposite directions, one naturally tended to counteract the other. This may account for the slower rate of closure of sternal rudiments *in vitro* and also for the fact that a certain minimal quantity of the dorso-lateral tissue had to be included in the explant, otherwise the movement towards the mid-line was completely cancelled by the outward migration of the peripheral tissue. It may be recalled, however, that when the sternal rudiments were cultivated in complete absence of the rib tips, the rate of closure was higher and did not seem to be reduced by the smaller amount of dorso-lateral tissue. The possible explanation is either that the material used in the present experiments was from younger embryos and was probably more sensitive to abnormal influences, or that the additional weight of the ribs slowed down the rate of movement since any outgrowth from the perichondrium of the ribs would tend to anchor them in the plasma clot. However, so long as the quantity of lateral tissue was large enough to prevent the entire stoppage of medial tissue movement by the migration of the peripheral cells, the result of the experiment was not affected by variation in the amount of rib stumps, muscle tags and sternal rudiments included in the different explants.

Conclusion

There is a general movement of undifferentiated tissue in the ventral body-wall in a median direction, which can take place after the removal of nearly all the lateral, differentiated tissues.

(3) CELL MIGRATION IN RELATION TO THE MOVEMENT OF THE STERNAL RUDIMENTS

The following experiments were made to find whether the sternal rudiments can be displaced by the amoeboid migration of adjacent cells when shrinkage of the ventral body-wall is eliminated.

Material and methods

Embryos with sterna between stages 2 and 3 (about 13 days) were used. The ventro-lateral body-wall was dissected as free as possible of muscle and skin, as in the preceding experiments. To eliminate the force of traction caused by the shrinkage of the mid-ventral body-wall, the explant containing the sternal rudiments was cut in two along the mid-line, and the halves were cultivated with their costal margins facing inwards and medial edges facing outwards. Any displacement of the sternal rudiments observed *in vitro* should be then due entirely to the amoeboid movement of the neighbouring cells. Rib stumps of varying length were left attached to each rudiment and the amount of soft tissue remaining on the costal margin was also varied. The cultures were studied for 2 or 3 days and a few for 4 days without transplantation.

As controls, some of the explants were cut in half and the halves laid on the clot with the normal orientation, i.e. with the medial edges facing each other.

Owing to the initial contraction of the tissue, the two halves in both groups were found to have moved away from each other after 1 hr. incubation. They were pushed gently together again. Altogether twenty-eight explants, half of which were controls, were cultivated and observed in five different experiments.

Camera lucida drawings were made after 2 hr. incubation and twice a day thereafter. Photographs were also taken of a few cultures. After 2–3 days of incubation, the explants were fixed in acetic alcohol, stained with toluidin blue, carmalum or dilute haematoxylin, and mounted whole.

Results

All fourteen explants with the sternal rudiments orientated back to back moved apart (cf. Pl. 1, figs. 2*c*, 2*d*). Though the rate of movement differed a little in different cultures, none remained stationary. In the fourteen control cultures, on the other hand, the sternal rudiments moved towards each other in a medial direction (cf. Pl. 1, figs. 2*a*, 2*b*); in a few the two sternal bands partially fused, while in the rest, they approached each other but did not unite within the period of observation.

In the explants with the medial cut edge facing outwards, there was initial shrinkage and the usual outgrowth of marginal cells into the medium. After 16 hr. cultivation, the two sternal bands had begun to move apart in all the explants, and a space could be observed between the two halves, occupied by a thin sheet of connective tissue cells. The movement was much faster during the second day so that the space became much wider and in a few cultures was invaded by the elongating rib stumps. There appeared to be no correlation between the amount of rib and connective tissue between the two sternal bands and the distance that the latter travelled. Camera lucida drawings showed that the rate of movement was approximately the same in all cultures, and the separation of the sternal bands evidently was not the result of the elongation of the ribs or the growth of the soft tissues.

When first explanted, the controls showed the usual initial contraction, and the marginal cells soon began to wander out. The cut edges of the two halves remained in contact with each other in most cultures, but in a few there were small gaps between the two halves, due to irregularities of the cut edges, which were bridged by connective tissue cells. The medial movement of the sternal bands was noticed after 16 hr. cultivation and had become more pronounced by the end of 24 hr. The two sternal bands were very close together after 48 hr. and partial fusion could be observed in a few explants. The rate of sternal closure was less than that usually observed *in vitro*, however, presumably due to additional disturbance caused by the median cut.

Conclusion

The sternal bands can be displaced by the amoeboid migration of neighbouring cells when shrinkage of the ventral body-wall is eliminated.

B. THE SEGMENTATION OF THE STERNUM

TECHNIQUE

The tissue culture technique previously described (Chen, 1952*b*) was used.

The explants were made of embryonic sterna at stage 3 of development (Chen, 1952*a*), i.e. the fusion of the sternal bands was not complete and hypertrophy of cartilage cells had not begun. The lateral and ventral body-wall containing the sternal rudiments and the rib stumps was dissected out. To ascertain the influence of the rib tissue on chondroblastic hypertrophy, the number of rib stumps left attached to the sternal bands was varied and the subsequent development *in vitro* of the latter was studied.

Experiments were also made in which other living tissues or glass capillaries were substituted for rib stumps, or the rib tissue was extracted in various ways in the hope of obtaining the active factors. The experimentally treated sternal rudiments, to which either substituted tissues were attached, or rib extracts were applied, were then cultivated by the watch-glass method and the effect of the treatment was studied.

Most of the explants were under observation for about 6 days and a few for slightly longer periods. Camera lucida drawings of the rudiments were made at intervals. The explants were fixed and sectioned as described before (Chen, 1952*b*).

EXPERIMENTS AND RESULTS

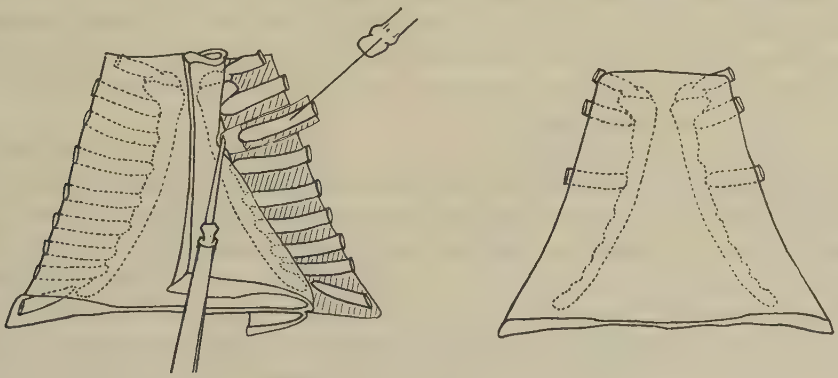
(1) THE INHIBITORY EFFECT OF THE RIBS ON CHONDROBLASTIC HYPERTROPHY

Series 1. *The development of the sternal rudiments in the complete absence of ribs*

Material and methods

These experiments have already been mentioned briefly in section A (p. 131) in connexion with the closure of the bands. From the point of view of the segmentation of the sternum, the reliability of the result depended on the completeness with which the ribs were removed. As described in the section on normal development (Chen, 1952*a*), sternal rudiments at the stage used for these experiments were intimately associated with the rib tips which appeared to have pushed into the sternal tissue. It was obvious, therefore, that by simply cutting along the two sides of the sternal rudiments, the small amount of rib tissue embedded in the sternal rudiments would be left behind. On the other hand, by passing the knife into the sternal tissue and around the contour of the rib tips, the latter could be removed, but the sternal tissue was almost always damaged. After many trials, complete removal of the ribs without injury to the sternal tissue was accomplished in the following way:

The piece of ventral body-wall including ribs and sternum was prepared in the usual manner. After complete or partial removal of the skin, the muscles and the connective tissue above and below the ribs were loosened with the aid of a fine knife and needle. They were then raised and pushed gently towards the sternal rudiment with the blunt edge of the knife until the junctions of the sternum and ribs were exposed. While they were held steadily in this position with the blade of a knife (see diagram, Text-fig. 1), the intercostal muscles were severed and one by one the ribs were pulled gently away from the sternal rudiment until they were disconnected.



Text-fig. 1. Diagram to show (*a*) the method of removing rib stumps from the sternal rudiment, (*b*) the resulting explant.

The explant was then cleaned of the muscle and connective tissue close to the sternal rudiments. An explant thus prepared suffered the least possible damage to its sternal tissue and yet was certainly freed from any trace of rib tissue. It consisted only of the ventral body-wall and the sternal rudiments which had a wavy outline, being narrower in the regions of the sterno-costal junctions than in the intercostal areas.

Eleven explants of stage 3 in normal development were isolated and cultivated; three served as controls with short rib stumps left attached to the sternal rudiments and from eight the ribs were completely removed.

Results

The results were completely consistent in that all the controls developed segmental hypertrophy while in the experimental explants the cartilage cells became hypertrophic throughout the entire length of the sternum except at the anterior end (i.e. the presternum).

During the first few hours of cultivation, the only obvious change was in the experimental group in which the sternal rudiments lost their initial wavy appearance and became uniform in width and smooth in outline. Subsequent changes during the first 24 hr. *in vitro* were the same in the cultures of both groups, consisting mainly of the medial movement of the sternal bands which were very close together. The sternal bands without rib stumps moved slightly faster and by the second day of cultivation were nearer to each other than those in the controls.

By the third day *in vitro*, obvious differences had appeared between the two sets of explants. While no sign of enlarged cells could be found in the controls, all the explants without rib tissue developed chondroblastic hypertrophy almost simultaneously in positions equivalent to the succeeding intercostal segments. During the next 24 hr., hypertrophy spread quickly through the whole length of the sternum. Sections of such a culture fixed on the fifth day (Pl. 2, figs. 3*a*, 3*b*) showed that except for the presternum which does not ossify, the whole sternum consisted of large vacuolated cells with basophilic matrix between them. The small-celled presternum was separated from the rest of the sternum by layers of flattened cartilage cells like those found at the distal zone of a normal hypertrophic area. No sign of segmentation was detectable in such a preparation. Periosteal bone-formation had also begun, and both the periosteum and bone collar developed as an uninterrupted sheet around the hypertrophic core of cartilage.

Five explants of the experimental group and one of the control set were fixed on the sixth day. In addition to the changes described above, excavation of the cartilage had begun in the experimental explants, as usual in the foremost region of the rudiments. Control explants, on the other hand, behaved exactly like those described before (Chen, 1952*b*); they were mainly small-celled with perhaps an early sign of hypertrophy in the first segment only. Longer cultivation of the control explants resulted, as already described, in segmental hypertrophy in the intercostal regions.

Two explants, free of rib tissue, which were cultivated for 25 days, showed that ossification took place normally; a thick, continuous layer of bone was deposited and large cavities filled with bone marrow elements were excavated in the hypertrophic cartilage (Pl. 2, fig. 4). The sternum thus appeared as a non-segmented bony structure with an anterior cartilaginous end.

Conclusions

(1) The removal of the rib tissue resulted in the absence of segmentation of the sternum.

(2) Closure and differentiation of the sternal rudiments were more rapid in the absence of the rib tissue.

Series 2. The development of sternal rudiments with ribs on one side only

Material and methods

Four sternal rudiments of stage 3 were dissected out, and the rib stumps on one side were removed according to the method described above. Seven short rib stumps on the other side were left attached.

Results

For the first 48 hr., the explants grew and developed as described in series 1, and the rate of closure was almost the same as in explants with no ribs. Although the fusion of the two sternal bands was not quite complete, hypertrophy began on the third day but it was much more advanced on the side with no rib stumps than on the other side to which ribs were attached. Sections of the explants fixed after

7 days of cultivation showed this difference clearly (Pl. 2, figs. 5*a*, 5*b*). On the side with ribs, hypertrophy was distinct only in the first segment while the cells in the following segments were just beginning to enlarge; the sternocostal regions were small-celled with the usual characteristic cell arrangement around the rib tips, so that the segmentation of this half of the sternal rudiment was distinct. On the side with no rib tissue, however, hypertrophy occurred throughout the whole length of the cartilage and the large vacuolated cells were uniformly distributed.

Periosteal bone-formation had begun by this stage and again was more advanced on the side without ribs (Pl. 2, fig. 5*b*). Fusion was not quite complete and the two bands were separated for the posterior two-thirds of their length by a thin layer of bony tissue which clearly belonged to the side with no ribs. The two-layered structure of the periosteum was quite distinct and the osteoblastic layer next to the very hypertrophic cartilage cells extended throughout the length of that half of the sternal rudiment.

Conclusions

(1) The results further demonstrated the inhibitory effect of the rib tissue on the segmentation and differentiation of the sternum.

(2) Such an effect was limited to the side to which the rib stumps were attached.

Series 3. The development of sternal rudiments with a varying number of rib stumps attached

Material and methods

Six sternal rudiments were dissected out. Instead of removing all the rib stumps, a few were deliberately left attached to each rudiment, the number and position of the attached rib stumps being varied in each explant.

Results

The results agreed with those already obtained.

When only the first, third and fifth pairs of ribs were retained, by the fifth day *in vitro* the sternal rudiment formed only three segments, besides the anterior cartilaginous portion (Pl. 2, figs. 6*a*, 6*b*). The first segment occupied a position equivalent to the normal first and second intercostal segments, the second included what would normally have been the third and fourth segments and the last segment was just below the level of the attachment of the fifth rib. In other words, hypertrophy was only inhibited in the areas to which a rib stump was attached. That these three new segments were the result of the coalition of the first and second, the third and fourth, and the fifth and sixth segments respectively, was clearly shown by the initial appearance of two adjacent centres of hypertrophy and later of ossification, in each of the three segments. At the sterno-costal junctions where the first, third and fifth pairs of rib stumps were attached, the arrangement of the cells was exactly the same as in a normal sterno-costal junction (Pl. 2, figs. 6*a*, 6*b*).

Other explants in which uneven numbers of rib stumps were left on the two sides of the sternal rudiments, confirmed this result (Pl. 3, figs. 7, 8). Wherever rib stumps remained, hypertrophy of the sternal cartilage cells was inhibited, the inhibitory effect of each rib extending only to that half of the sternal tissue to which it was attached. Thus alternate areas of hypertrophy appeared, bilaterally different according to the number of rib stumps retained on each side.

Conclusion

The results supported those obtained in other experiments in showing that each rib stump exerted its inhibitory effect on those cartilage cells of the sternum that were immediately next to it.

Series 4. The effect on sternal development of adding extra rib stumps to the rudiment

Material and methods

To implant the extra rib stumps on the sternal rudiments, the latter were first dissected free of all except two pairs of rib stumps, usually the first and third, the presence of which helped to stabilize the position of the implants. The latter, either two or three pairs, were taken at random from among the detached rib stumps and implanted between the attachments of the two pairs of retained rib stumps. Five explants were thus prepared and cultivated.

Results

After 5 or 6 days *in vitro* all the sternal rudiments had hypertrophied in the area below the attachment of the third rib, where all the ribs had been removed, while the first and third sterno-costal junctions remained small-celled. The situation in the area corresponding to the normal first and second intercostal segments, i.e. where the two extra rib stumps were implanted, varied in different explants. In two, the effect of the implanted rib stumps was obvious; hypertrophy was interrupted where the added ribs were attached, and was therefore restricted to a small area just below the first rib (Pl. 3, figs. 9*a*, 9*b*). In other words, the two pairs of added rib stumps acted in the same way as the normally attached second pair, only with perhaps greater strength. In two other explants, the effect of the added rib stumps was not so clearly demonstrated; the hypertrophy was only partially inhibited in the area of attachment, as shown by the persistence of smaller cells immediately next to the tips of the ribs.

In the remaining explants, added ribs seemed to have no influence on the sternum, which developed hypertrophy throughout the area between the attachment of the rib stumps originally present, i.e. the first and third pairs.

The discrepancy in these results was readily explained upon careful examination of the sections of these explants. It was found that, although the same method of inserting the rib stumps was employed in all experiments, the degree to which these added stumps became attached to the sternum varied in different explants, and only when the contact between the two tissues was well established could any effect of the implanted ribs be seen. When inhibition was observed, the periosteum of the sternal tissue was interrupted at the place of attachment and the rib appeared to have penetrated into the sternal tissue. When the effect of rib tissue was not clearly shown, the stumps were only attached to the periosteum of the sternum and were not in intimate contact with the sternal tissue itself.

Conclusion

Added rib stumps exerted the same effect as those naturally present, provided that intimate contact with the sternal tissue was established.

Series 5. The effect of loosening the rib stumps from the sternal rudiment

Material and methods

The latero-ventral body-wall containing the sternal rudiments attached to the rib stumps was dissected from 14-day embryos. Instead of being removed, the ribs were pulled away and loosened from the sternal rudiments to different extents and were then cultivated. Usually only the second, third and fourth pairs of rib stumps were thus loosened, the others being left untouched. Controls with intact rib stumps were also cultivated for comparison.

Results

The control explants developed the usual segmental hypertrophy and ossification of cartilage cells, but in the sternal rudiments with loosened rib stumps, consecutive intercostal areas of hypertrophy showed a varying degree of coalescence across the sterno-costal junctions where the rib stumps had been loosened. In other words, by breaking the contact between the sternal and rib tissue, the inhibitory effect of the latter was reduced.

For the first few days of cultivation, the sternal rudiments in both groups developed in the same way as those described previously (Chen, 1952*b*); they underwent fusion and chondrification and no significant difference between the two sets could be seen. After 5 days *in vitro* the cartilage cells had hypertrophied in the first three or four segments in both groups. But instead of the uniformly clear-cut picture of alternating areas of enlarged cells (the intercostal regions) and of small cells (the sterno-costal junctions) present in the control explants, in the rudiments of the experimental group, some sterno-costal junctions were not clearly distinguishable from the neighbouring intercostal regions. The difference between the two series was even more evident in the later stages of development, and after 7 days' cultivation, several areas of hypertrophy had coalesced in explants of the experimental group so that the normal pattern of segmentation was disturbed. There was a bilateral independence in the coalescence which was apparently correlated with the degree of displacement of the rib stumps concerned.

The effect of different degrees of displacement was well illustrated in sections of explants fixed after 10 days' cultivation (Pl. 4, figs. 10*a*, 10*b*, 11*a*, 11*b*, 12*a*, 12*b*). The most seriously affected ribs were pulled right out of the sternal tissue which had deposited a continuous bone collar between the end of the rib and the sternum (Pl. 4, figs. 12*a*, 12*b*). Under these conditions the cells in the sternum next to the attachment of the rib were enlarged and no trace of small cells was left. In the least affected rib stumps (Pl. 4, figs. 11*a*, 11*b*) the ends were still embedded in the sternal tissue, the cells next to the tips of the rib remaining small. The cells in the interior of the sternum, however, were enlarged and in this area there was no distinction between the sterno-costal and the adjacent intercostal region. In other words, the layer of compressed cartilage cells normally present between an intercostal region and a sterno-costal junction was absent, so that the enlarged cells of the intercostal region seemed to be spreading across the sterno-costal junction to the next intercostal region. In the intermediate cases, the tips of the rib stumps were not embedded in the sternal tissue but only pierced the periosteum, so that the formation of the bone collar was interrupted to a varying extent at the point of attachment. Small cartilage cells were only present immediately next to the rib tips and most of the sterno-costal region consisted of enlarged cells (Pl. 4, figs. 12*a*, 12*b*).

Thus, while the result of loosening the rib stumps appeared variable in the living cultures, histological studies showed that the pulling displaced individual rib stumps to a varying extent, and that the inhibitory effect of the ribs on hypertrophy was reduced according to the degree to which the rib stumps were shifted.

Conclusion

Normal contact between the rib and the sternal rudiment is at least one important factor responsible for the inhibitory effect of the ribs on the hypertrophy of the sternal cartilage cells.

(2) PRELIMINARY ATTEMPTS TO STUDY THE MECHANISM OF THE INHIBITION

Since the experiments reported above demonstrated beyond doubt that rib stumps exerted an inhibitory effect on the hypertrophy of the cartilage cells of the sternum, it seemed interesting to investigate the nature of this effect.

Preliminary attempts were therefore made to find (1) whether the action is specific in that it is exerted only by the rib tissue; and (2) whether it is predominantly mechanical or chemical in nature.

Series 1. *The effect of other tissues or materials on the hypertrophy of the cartilage cells of the sternum*

Material and methods

Sternal rudiments were dissected free of all except the first and third pairs of rib stumps. A short piece of clavicle of the same embryo, part of a sternal band from a litter-mate, or two or three fine glass capillaries were inserted between the first and third pairs of ribs in the same way as an extra rib stump was implanted in earlier experiments.

Results

In all explants, these devices failed to inhibit hypertrophy even when intimate contact with the sternal rudiment was obtained.

Three explants on which clavicle stumps had been implanted were fixed after 5, 7 and 11 days' cultivation respectively. After 5 days *in vitro* the cartilage cells hypertrophied throughout the length of the sternum, except where the first and third pairs of rib stumps were attached (Pl. 5, fig. 13). The clavicles, which themselves were ossified, were well grafted to the sternal tissue, the fibroblastic layer of their periosteum being continuous with that of the sternum. The intimate contact between the two tissues was more clearly demonstrated when the sternum began to ossify at the end of the seventh day *in vitro*, for the bone collar of the latter was formed in continuity with the osseous tissue of the clavicle. Nevertheless, there was no evidence that any pressure was exerted by the grafted tissue on the sternum.

Two explants on which other sternal rudiments had been grafted were cultivated for 6 and 7 days respectively. Again the contact between the implanted rudiment and the 'host' sternum was good so that no demarcation between the two was discernible. Hypertrophy was well developed on the sixth day in both the 'host' and the graft, and ossification began on the seventh day. As in the experiment with clavicle stumps, the periosteal bone collars that were formed in the two rudiments were continuous with each other.

In one of the two explants on which glass capillaries were implanted, the three pairs of capillaries were in contact with the fibroblastic layer only, while in the other they were inserted into the sternal tissue in different degrees, so that in some places, both layers of the periosteum were interrupted and in others, only the fibroblastic layer was penetrated. In this explant alone, the close contact of the glass capillaries with the sternal tissue seemed to have produced some effect on the cartilage cells, which in sections appeared to be under pressure and were less hypertrophic than those below the attachment of the third ribs (Pl. 5, fig. 14).

Conclusion

Under the conditions of the present experiments other tissues and substances do not exert the same effect as ribs in inhibiting hypertrophy of the chondroblasts.

Series 2. *Experiments to detect the presence of a chemical substance in the rib tissue responsible for its inhibitory effect*

Material and methods

Before explantation, the sternal rudiments were either freed of all rib stumps or only the first and third pairs were left. In the different experiments one of the following methods was adopted:—(*a*) An attempt to isolate the active factor in the rib tissue was made by extracting the rib stumps with either Tyrode solution, or acetone; the latter was evaporated to dryness and the residue was reconstituted with Tyrode solution. The extract was added to the culture medium. Fourteen explants were cultivated in this way. (*b*) Isolated rib stumps were cultivated by themselves for 3 days and the exudate from such a culture was collected and deposited on explants of sternal rudiments. Two explants were cultivated. (*c*) Rib stumps were ground and mixed with 5 % agar solution or cock's plasma. The solidified mixture was then cut into thin small slices and planted on to three sternal rudiments in place of rib stumps. (*d*) To see whether the dead rib could exert the same inhibitory effect, rib stumps were first boiled in Tyrode solution for 15 min. and then implanted on a sternal rudiment, as previously described. Two explants were cultivated.

Results

All the experiments gave a negative result. The sternal rudiments were not affected by the rib exudate or rib extract that was added to the cultures, and hypertrophy of cartilage cells occurred throughout the whole length of sternum in these explants. In sternal rudiments on which were implanted boiled rib stumps (Pl. 5, fig. 15), agar (Pl. 5, fig. 17) or plasma clot (Pl. 5, fig. 16) containing minced rib tissue, hypertrophy was inhibited only where the naturally attached rib stumps were retained, i.e. at the first and third sterno-costal junctions. In the area between these two regions the cells showed no signs of being affected by the implanted elements, and by the end of 6 or 7 days the chondroblasts were very hypertrophic. The contact between the implanted elements and the sternal rudiments was better with plasma clot than with agar gel and boiled rib stumps.

Conclusion

By the methods used, it was not possible to detect any chemical substance that may be responsible for the inhibitory action of the rib stumps.

DISCUSSION

(1) The closure of the sternal bands

The first series of experiments recorded in section A (1) showed that the sternal bands were not mechanically pushed together by the elongation of the ribs, since closure took place in the complete absence of the ribs.

The second set of experiments indicated that there is a movement of undifferentiated tissue in the ventral body-wall towards the mid-line, which proceeds independently of the differentiated lateral tissues, viz. pectoral muscles, ribs, and so forth.

That this movement is active, and due to the amoeboid migration of the component cells rather than to the spontaneous shrinkage of the mid-ventral body-wall, has been shown by the third set of experiments, which demonstrated that the sternal bands can be displaced in the absence of any traction due to shrinkage, since they moved apart when explanted back to back. The argument that the bands might be forced apart by growth of the intervening tissue is unfounded, since the distance through which the bands moved was quite unrelated to the amount of tissue connecting them; the quantity of tissue left attached to the dorsal borders of the bands was purposely varied within wide limits to test this point.

It therefore appears that the primary factor in causing the union of the sternal bands is the streaming of undifferentiated cells in a ventral direction. The shrinkage and degeneration of the mid-ventral body-wall, on the other hand, which may play a part in the closure under normal conditions, is not essential and may well be only a secondary factor.

In short, the results agree with those reported on the avian sternum (Fell, 1939), and the mechanism for the closure of the sternal bands is thus the same for both mammals and birds.

(2) The segmentation of the sternum

Although it has been shown that the mouse sternum is largely self-differentiating, its segmentation, like the formation of some of the structural details of other bones, is not controlled by intrinsic factors. As the experiments on the removal of the ribs have clearly demonstrated, segmentation depends on the influence of the ribs. It is not due to the displacement and obliteration of sternal tissue by the ingrowth of the ribs, for the study of the normal development of the sternum (Chen, 1952*a*) has shown that the sternal tissue persists between the opposing rib tips, but the cells of the intervening cartilage fail to enlarge. Thus the ribs inhibit chondroblastic hypertrophy in the sternal tissue, but the inhibitory effect is restricted to the cells immediately next to the rib tips, is therefore quite localized and can be produced by the addition of any extra rib stumps.

While the presence of an inhibitory effect is undoubted, how it is produced remains obscure, and the preliminary experiments reported above provided no conclusive evidence as to whether it is due to chemical or mechanical factors or to both.

The possible existence of a chemical factor is suggested by the fact that the sternal ends of the ribs which cause the inhibition are themselves not ossifiable. It is interesting to note that although most embryonic cartilages are replaced by bone in endochondral ossification, there are a few, such as those forming the ventral ends of the ribs and in the respiratory passages, which remain unossified in adult life. The reason for this is unknown, and since the essential biochemical changes accompanying the replacement of cartilage by bone are still obscure, it is impossible to tell what delays or prevents a cartilage from undergoing endochondral ossification. Nevertheless, it seems logical to assume that some essential difference exists between cartilage that is ossifiable and that which remains unossified, such as the lack of some essential substance for ossification or the presence of an inhibitory factor. Thus in the present case, the non-ossifying costal cartilage may possess some substance which inhibits hypertrophy and ossification not only in itself, but also in the adjacent sternal cartilage. However, if this is so, the chemical factor is difficult to detect under our experimental conditions, since all attempts to extract it proved futile. Obviously the negative results may indicate either the absence of a chemical agent, or that the substance is very labile and depends on the intactness of the tissue for its normal functioning. No conclusion is therefore possible at the present stage.

Alternately, the view that the inhibitory effect on the hypertrophy of sternal cells may be mechanical, is quite plausible because the ribs elongate considerably during development and there is evidence that the sternal tissue between opposing rib tips is under pressure at a certain stage of development (Chen, 1952*a*). The possible effect of the elongation of the ribs on segmentation has been indirectly suggested by Bryson (1945) who found that in the screw-tail mouse, whose sternum is broad and not fully segmented, the distance between the approaching ends of the ribs of either side is also abnormally great, due to a slow rate of growth of the ribs.

As described above, when extra rib stumps were implanted they too exerted an inhibitory effect on ossification, provided that an intimate contact with the sternal tissue was successfully established. The importance of a normal contact was also significantly illustrated when the loosening of rib stumps resulted in the decrease or loss of the inhibitory effect. Such experimental facts again emphasize the delicate nature of the factor or factors responsible for the inhibition of ossification, but are perhaps more readily explained on a mechanical than a chemical basis.

On the other hand, if the effect of the ribs were purely mechanical, it might be expected that a well-grafted embryonic clavicle or sternal band would exert some influence due to their growth in length, but these living structures had no more effect on the hypertrophy of the sternal tissue than did implanted glass capillaries. These negative results seem to imply a qualitative difference between these rudiments and the sternal ends of the ribs, in that the former are ossifiable, whilst the latter are not; this again indicates the possible participation of a chemical factor.

The problem therefore remains open, and it is hoped that further work will be done in this connexion.

SUMMARY

(1) THE CLOSURE OF THE STERNAL BANDS

The factors responsible for the closure of the sternal rudiments were investigated.

The sternal bars moved together in the complete absence of the ribs.

The differentiated tissues including the clavicles and pectoral muscles were almost completely removed from the ventral body-wall which was cultivated *in vitro*; the movement of the tissue was followed by carbon marks placed on the skin just within either lateral border. The explants increased in circumference but the marks moved towards the mid-line.

Carbon marks on sheets of body-wall from the costal region which were cultivated as controls, moved apart.

It was thus shown that a general movement of tissue in a median direction occurs in the ventral body-wall and that this movement does not depend on the presence of the differentiated tissues.

When the ventro-lateral body-wall was cut in half longitudinally and cultivated with the dorsal margins of the two halves facing inwards and their ventral margins facing outwards, the sternal bands became united dorsally by a sheet of connective tissue but moved apart instead of approaching each other. In controls in which the two halves of the ventro-lateral body-wall were arranged in their normal orientation, i.e. with their ventral margins facing inwards, the halves united and the bands moved together as usual.

The results showed that the sternal bands can be displaced by the amoeboid migration of adjacent cells.

It was concluded that the closure of the sternal bands is due primarily to the active streaming of undifferentiated cells towards the mid-line. The shrinkage and degeneration in the mid-ventral body-wall probably plays only a secondary role.

(2) THE SEGMENTATION OF THE STERNUM

The effect of the ribs on the segmentation of the sternum was investigated experimentally.

A method was devised to ensure the complete removal of rib tissue from the sternum at each sterno-costal junction.

In the complete absence of all rib stumps, the sternum failed to develop segmentation; there was no persistence of small-celled cartilage and hypertrophy occurred throughout the length of the sternum.

When only a few of the rib stumps were removed, small-celled cartilage remained only where rib stumps were left attached.

The results showed that the effect of the rib stumps was to inhibit the hypertrophy of the cartilage cells adjacent to them.

This inhibitory effect was unilaterally localized and was limited to cells immediately next to the rib tissue.

Extra rib stumps implanted on the sternal tissue exerted the same effect as those naturally attached.

When the rib stumps were merely loosened instead of removed from the sternal tissue the inhibitory effect was lost to an extent that depended on the degree of detachment.

Preliminary studies were made to ascertain the nature of the inhibitory effect of the rib tissue.

Attempts to extract an active chemical factor from the rib tissue were not successful.

The effect of ribs could not be imitated by other tissues or substances.

The possible nature of the inhibitory effect was discussed.

REFERENCES

Bryson, V. (1945). Development of the sternum in screw-tail mice. *Anat. Rec.* **91**, 119–138.

Chen, J. M. (1952*a*). Studies on the morphogenesis of the mouse sternum. I. Normal embryonic development. *J. Anat., Lond.*, **86**, 373–386.

Chen, J. M. (1952*b*). Studies on the morphogenesis of the mouse sternum. II. Experiments on the origin of the sternum and its capacity for self-differentiation *in vitro*. *J. Anat., Lond.*, **86**, 387–401.

Fell, Honor B. (1939). The origin and developmental mechanics of the avian sternum. *Phil. Trans.* B, **229**, 407–463.

Gladstone, R. J. & Wakeley, C. P. G. (1932). The morphology of the sternum and its relation to the ribs. *J. Anat., Lond.*, **66**, 508–564.

EXPLANATION OF PLATES

Abbreviations

ag	agar gel	*p*	periosteum
ai	absence of inhibition	*pc*	plasma clot
br	boiled rib	*pst*	presternum
cl	clavicle	r_1	first rib
es	empty space left by glass capillary	r_2	second rib
hc	hypertrophic cartilage	r_3	third rib
icl	implanted clavicle	r_4	fourth rib
ii	incomplete inhibition	r_5	fifth rib
		r_i	implanted rib

Plate 1

Fig. 1*a*. The ventral body-wall of a 13-day embryo, photographed 2 hr. after explantation. Four patches of gas black have been laid on the surface of the tissue. ×18.

Fig. 1*b*. Same explant (*a*) photographed after 48 hr. *in vitro*. Note that the four patches of gas black have moved to the mid-line. The outgrowth of tissue which is out of focus is not shown. ×18.

Fig. 1*c*. Section of the same explant (*b*), fixed after 2 days' growth. Note that the margins of the explant extends laterally far beyond the rib stumps (*rs*). Haematoxylin and chromotrope. ×55.

Fig. 1*d*. Explant of lateral body-wall from the same embryo and grown in the same watch-glass as the preceding explant of ventral body-wall. Four patches of gas black have been laid on the ectoderm. Photographed 2 hr. after explantation. ×18.

Fig. 1*e*. Same explant (*d*) after 48 hr. *in vitro*. The carbon marks have moved apart. ×18.

Fig. 1*f*. Section of the same explant (*e*) fixed after 2 days' growth. Haematoxylin and chromotrope. ×55.

Fig. 2. Photographs of two living cultures of the ventro-lateral body-wall of a 13-day embryo.

Fig. 2*a*. The body-wall has been cut in half down the mid-ventral line and the two halves have been arranged with the ventral margins of the sternal bands facing each other in the usual way. ×18.

Fig. 2*b*. The same explant as (*a*) after 48 hr. cultivation. The sternal bands have moved together. Carmalum. ×22.

Fig. 2*c*. Similar explant which has been cut in half but the two halves have been arranged with the costal margins of the sternal bands facing each other. (*a*) and (*c*) were photographed 2 hr. after explantation. × 18.

Fig. 2*d*. The same explant as (*c*) after 48 hr. cultivation. The costal margins are united by a sheet of connective tissue but the sternal bands have moved widely apart. Carmalum. × 22.

PLATE 2

Fig. 3*a*. Frontal section of a sternal rudiment from a 14-day embryo after 5 days' cultivation. All the ribs were completely removed before explantation. Hypertrophy of the cartilage cells and ossification have appeared throughout the whole length of the sternum; there is no trace of the normal segmentation. Haematoxylin and chromotrope. × 28.

Fig. 3*b*. Part of the same section enlarged. × 155.

Fig. 4. Frontal section of a sternal rudiment freed of all rib stumps from a 13½-day embryo; fixed after 25 days' cultivation. The non-segmented hypertrophic cartilage has been excavated and replaced by bone. Haematoxylin and chromotrope. × 46.

Fig. 5*a*. Frontal section of the sternal rudiment from a 14-day embryo, with rib stumps on one side only, after 7 days' cultivation. Unilateral chondroblastic hypertrophy is seen on the side without rib stumps and a continuous sheath of bone is also present on that side. The inhibitory effect of the ribs is clearly seen in the persistence of small-celled cartilage immediately next to them. Haematoxylin and chromotrope. × 21.

Fig. 5*b*. Part of the same section enlarged. × 120.

Fig. 6*a*. Frontal section of the sternal rudiment from a 13½-day embryo after 5 days *in vitro*. Only the first, the third and the fifth pair of ribs were retained. Chondroblastic hypertrophy and ossification have appeared in the region where the ribs were removed. Opposite the retained ribs the cartilage is small-celled. Haematoxylin and chromotrope. × 30.

Fig. 6*b*. Part of the same section enlarged. × 95.

PLATE 3

Fig. 7*a*. The sternal rudiment from a 14-day embryo after 5 days *in vitro*. Only the first and the third pairs of ribs were retained. Cell hypertrophy and ossification have appeared throughout the sternum except where the ribs were retained. Haematoxylin and chromotrope. × 28.

Fig. 7*b*. Part of the same section enlarged. × 112.

Fig. 8*a*. Sternal rudiment from a 13-day embryo after 5 days *in vitro*. Only the second rib on the right and the fourth rib on the left were retained. Hypertrophy of the cartilage cells and early ossification have appeared in the regions where the ribs were removed. Opposite the retained ribs the cartilage is small-celled. Haematoxylin and chromotrope. × 32.

Fig. 8*b*. Part of the same section enlarged. × 155.

Fig. 9*a*. A sternal rudiment from a 13-day embryo after 6 days *in vitro*. All but the first and third pairs of ribs were removed; two extra pairs of ribs were then planted in place of the second pair. Hypertrophy of the cartilage cells and ossification have appeared throughout the posterior part of the sternum, but hypertrophy is restricted to a small area in the anterior portion. Haematoxylin and chromotrope. × 20.

Fig. 9*b*. Part of the same section enlarged. The area affected by the addition of the extra ribs is shown. × 155.

PLATE 4

Fig. 10*a*. Frontal section of a control sternal rudiment from a 14-day embryo with all the rib stumps intact; 10 days' cultivation. Segmentation is normal. Haematoxylin and chromotrope × 28.

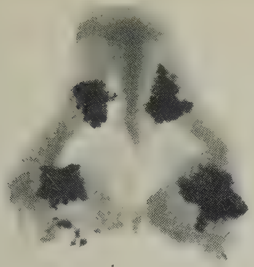
Fig. 10*b*. Part of the same section under higher magnification. × 88.

Fig. 11*a*. Frontal section of a similar explant in which the rib stumps were loosened; 10 days' cultivation. Segmentation is less complete than in fig. 10. Haematoxylin and chromotrope. × 28.

Fig. 11*b*. Part of the same section under higher magnification. × 88.

Fig. 12*a*. Frontal section of a similar explant in which the ribs were pulled away from the sternum; 10 days' cultivation. There is no segmentation. Haematoxylin and chromotrope. × 28.

Fig. 12*b*. Part of the same section under higher magnification. × 88.



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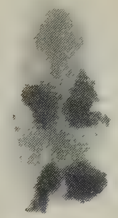

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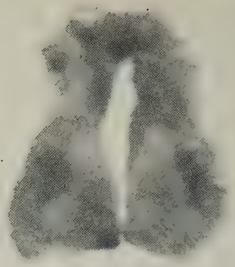

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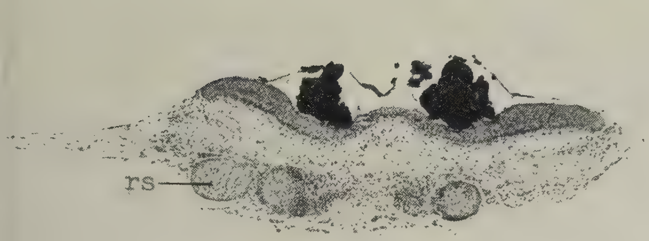

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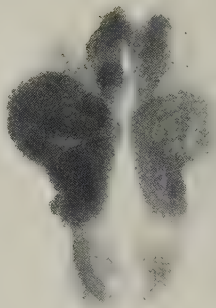

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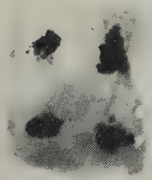

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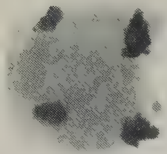

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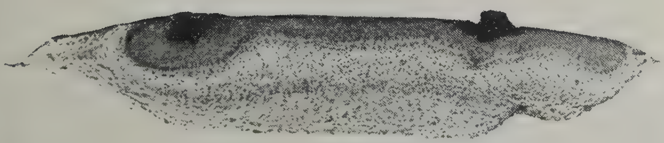

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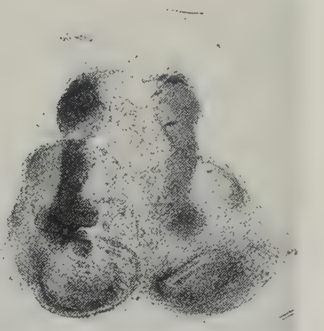

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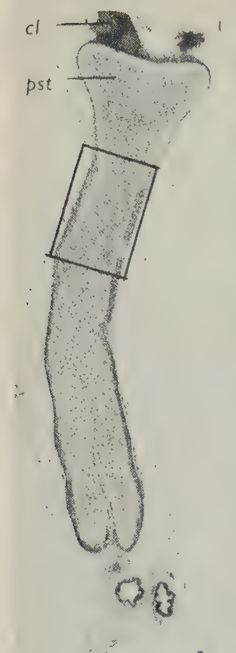
JUI MING CHEN. III.—Studies on the morphogenesis of the mouse sternum



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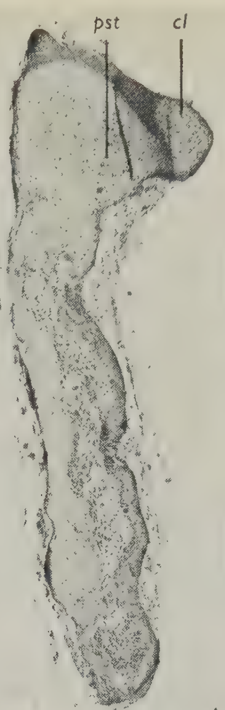

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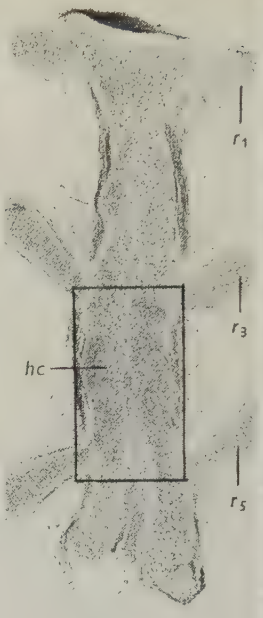

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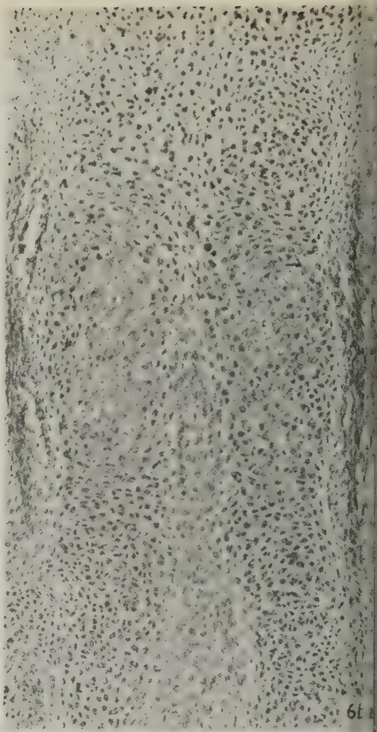

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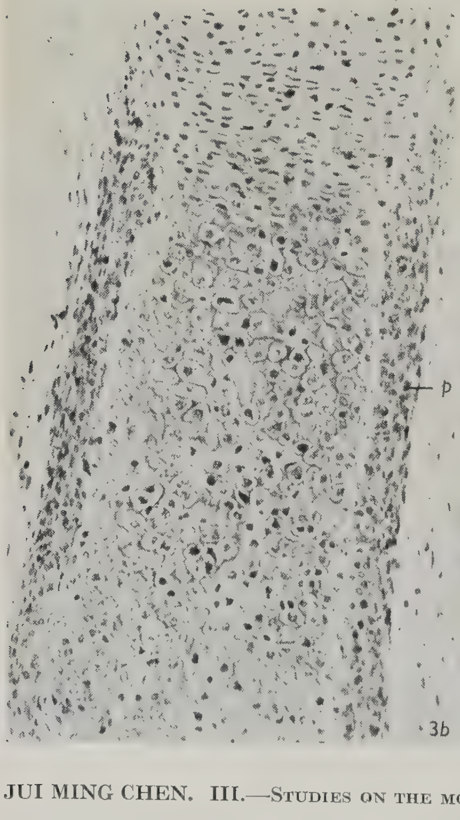

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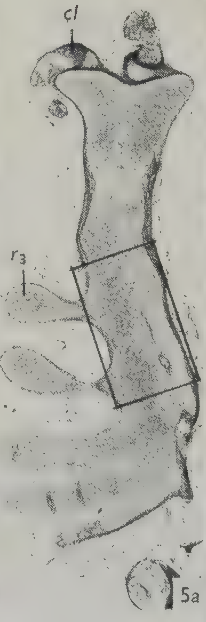

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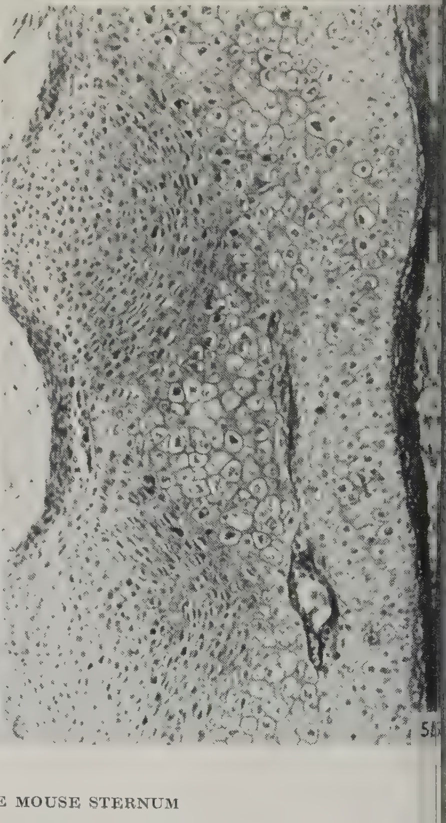

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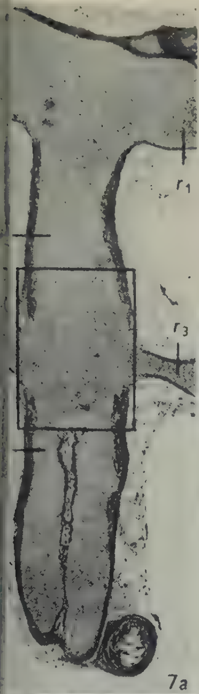
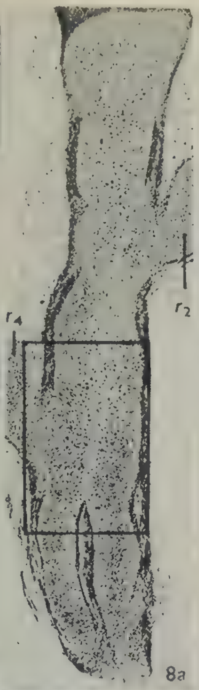
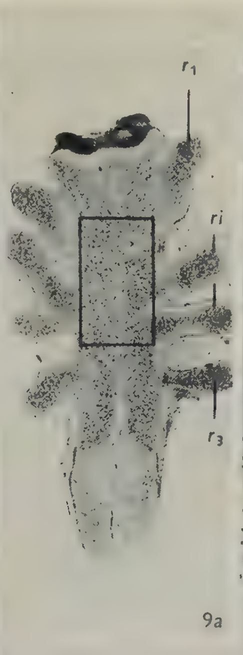
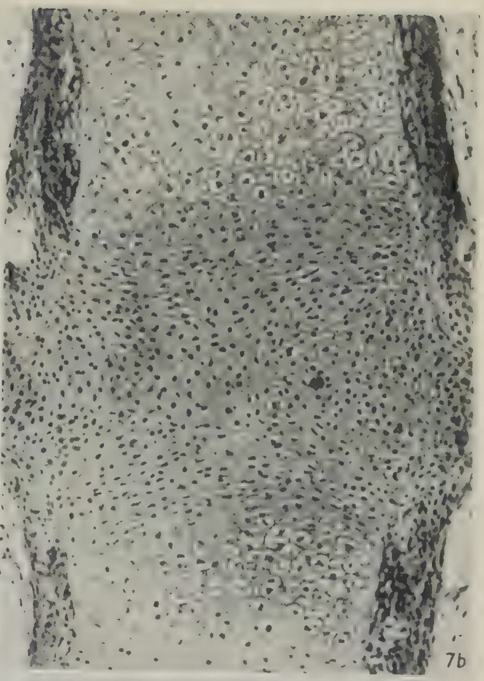
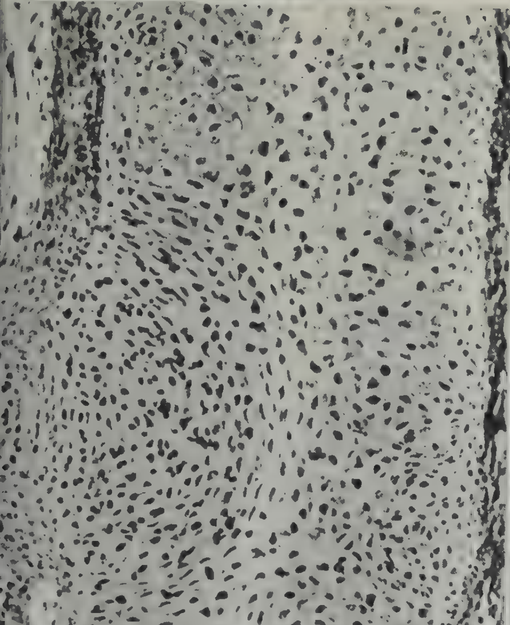
JUI MING CHEN. III.—STUDIES ON THE MORPHOGENESIS OF THE MOUSE STERNUM

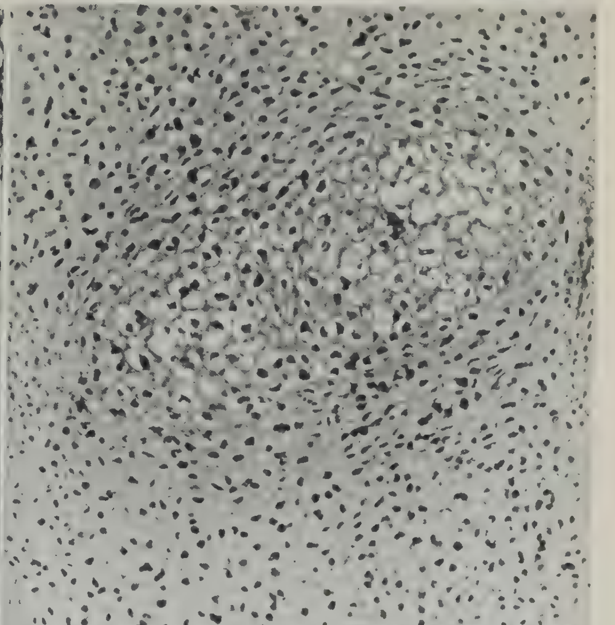




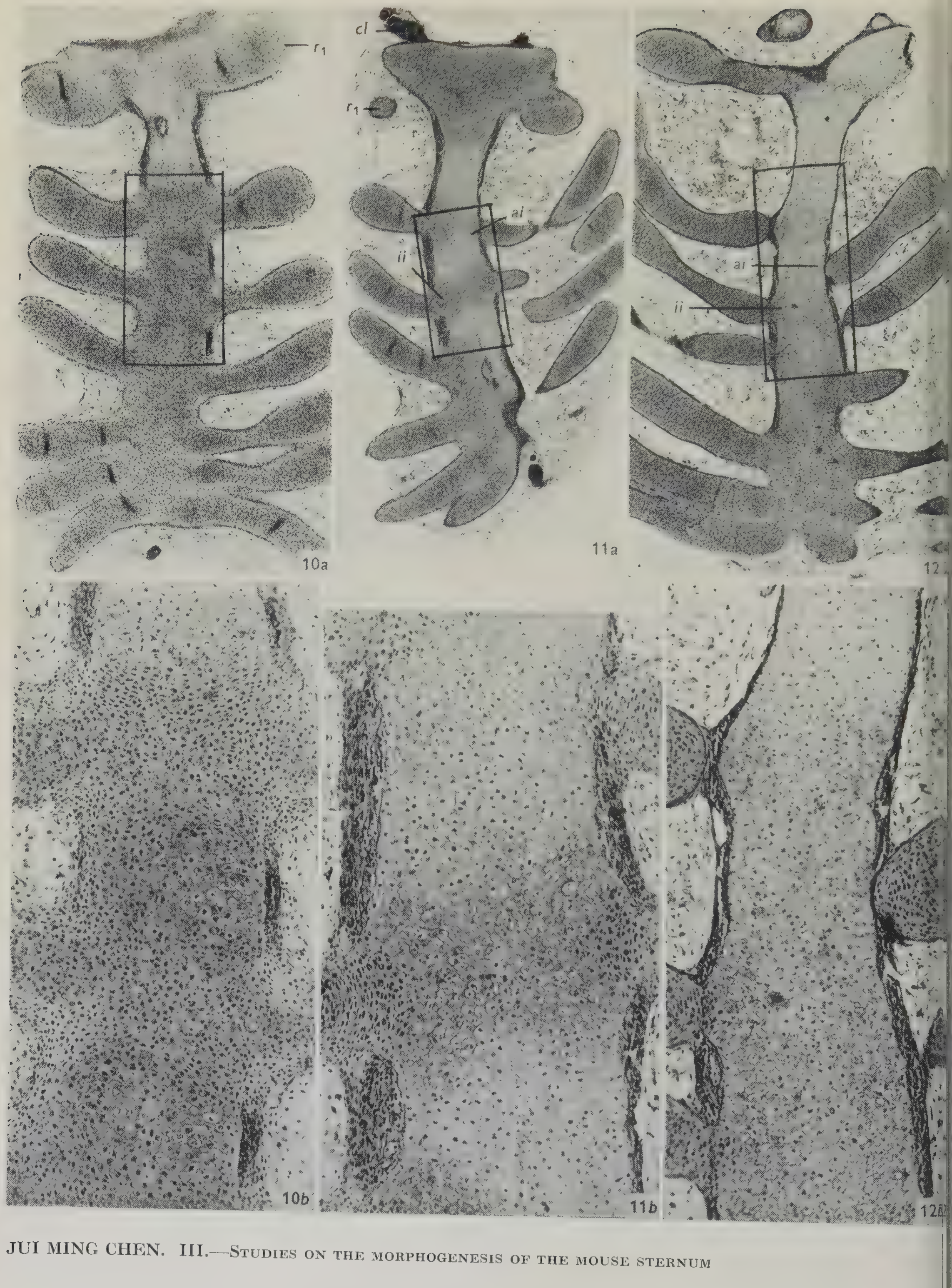








MING CHEN. III. Studies on the morphogenesis of the mouse sternum



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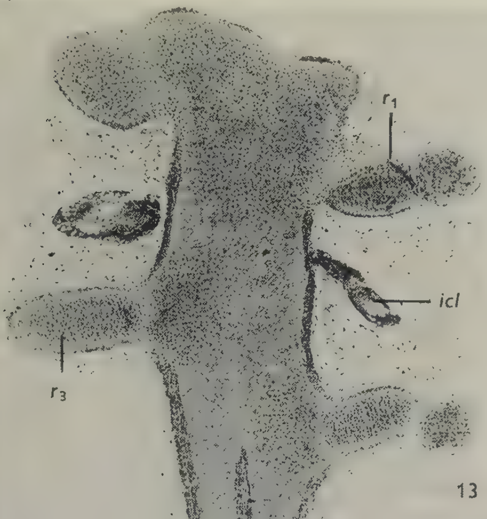

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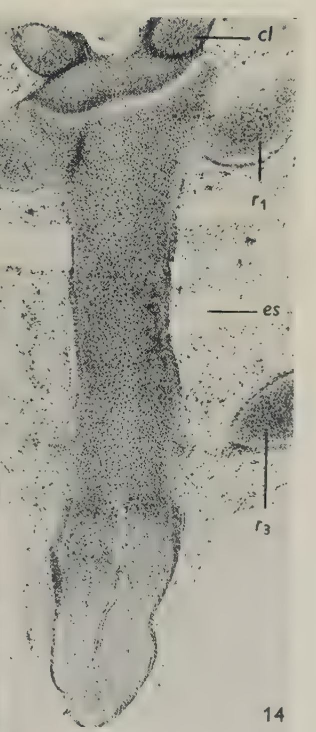

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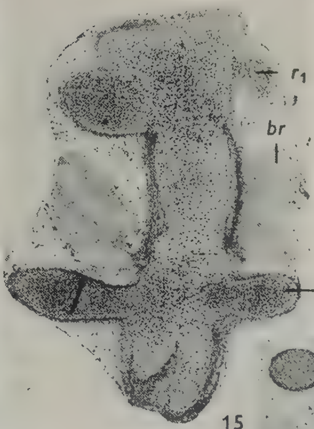

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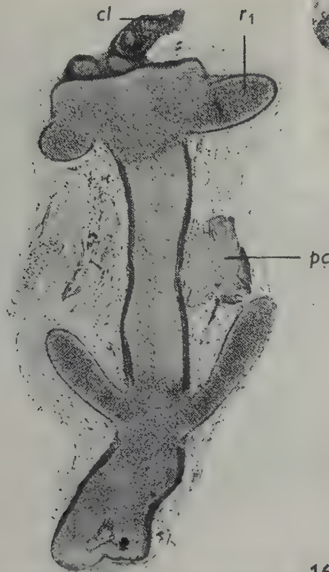

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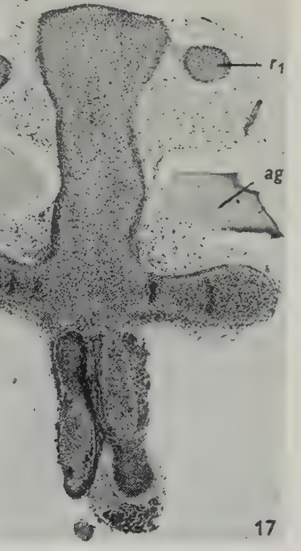

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JUI MING CHEN. III.—STUDIES ON THE MORPHOGENESIS OF THE MOUSE STERNUM

PLATE 5

Fig. 13. Frontal section of the sternal rudiment from a 14-day embryo after 5 days' cultivation; the first and third pairs of rib stumps were left attached and a pair of clavicle stumps were implanted between them. No interruption of hypertrophy is seen where the clavicle is attached. Haematoxylin and chromotrope. × 32.

Fig. 14. Frontal section of the sternal rudiment from a 14-day embryo after 5 days' cultivation; the first and third pairs of rib stumps were left and five glass capillaries, three on one side and two on the other, were implanted between them. The glass capillaries which were removed before the sections were cut, appear as empty spaces, one of which is not seen in this section. Although chondroblastic hypertrophy has occurred continuously in this region it is not so advanced as in the sternal cartilage below the third rib. Haematoxylin and chromotrope. × 33.

Fig. 15. Frontal section of a 14-day sternal rudiment after 6 days *in vitro*. All but the first and third pairs of ribs were removed and boiled rib stumps were implanted in contact with the sternum. These implants have not affected chondroblastic hypertrophy. Haematoxylin and chromotrope. × 40.

Fig. 16. Similar explant after 7 days *in vitro*. Plasma clot containing minced rib tissue was implanted on to sternum but produced no effect on cell hypertrophy. Haematoxylin and chromotrope. × 20.

Fig. 17. Similar explant after 8 days' cultivation. Agar gel containing rib extract was implanted on to the sternum; cell hypertrophy was not affected. Haematoxylin and chromotrope. × 20.

THE GROWTH OF EMBRYONIC NEURITES

A STUDY ON CULTURES OF CHICK NEURAL TISSUES

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INTRODUCTION

That the tip of an embryonic growing nerve fibre terminates in a distinct expansion was first recognized in 1890 by Ramón y Cajal, who gave the name of 'cone d'accroisement' thereto. The amoeboid behaviour of this structure was first described by Harrison in 1910, in those papers which not only definitely established the neurone theory of Ramón y Cajal but also founded the technique of tissue culture, and so opened a wholly new approach to the experimental study of cells and tissues in general. Thus research on the growth of neural tissues in culture dates from the very beginning of the use of such methods in experimental biology (Harrison, 1907, 1910; Burrows, 1911; Lewis & Lewis, 1912). Since that time, tissue-culture methods have been used for all but one of the studies on the behaviour of the living amoeboid tip, the exception being the brilliant observations of Speidel (1932, 1933) on the growth of neurites in the tail of living amphibian larvae. Levi (1934) has summarized the studies on explants of nervous tissues in his general survey of the results obtained by tissue-culture methods. Many of the observations on cultures of the nervous system have been made by Levi himself; in 1917, for instance, he published a series of drawings of the changes undergone during a period of 5 min. by the tip of a neurite in an explant of chick mid-brain.

A recent account of the behaviour of living nerve fibres in culture is due to Lewis (1950), who describes some time-lapse films of nerve cultures presented at a Symposium on *Genetic Neurology* in March 1949, though no illustrations from these films are included in the published record to console the reader for absence on this occasion. The use of phase-contrast microscopy for the study of cultures of nervous tissues has been introduced by Prof. C. M. Pomerat (Costero & Pomerat, 1951), who has prepared by this means a number of time-lapse films on the behaviour of cells from the adult nervous system.

In this paper some observations are described which have been made of the tips of living neurites emerging from explants of neural tissues of the embryonic chick.

The behaviour of these structures has been recorded by phase-contrast ciné-microscopy, and the conclusions which have been reached are derived from the analysis of the resulting film records.

TECHNIQUE

Explants of spinal ganglia, segments of the spinal cord, and fragments of the cerebral hemispheres or optic lobes were made from 7–12-day chick embryos under the usual conditions of tissue culture. These explants were grown as hanging-drop preparations, usually in a clot consisting of approximately equal parts of diluted embryo extract and adult fowl plasma. Some were grown in a fluid medium; this was obtained by first preparing a clot of embryo extract and plasma, then chopping it into small pieces from which an exudate drained slowly away. This fluid was incubated for a period of an hour together with the clot fragments and was then collected for use.

The cover-slips bearing the cultures were mounted on culture chambers suitable for phase-contrast microscopy, consisting of a metal ring 1–2 mm. in thickness, on the underside of which was sealed a further cover-slip of the same size. The study and photographic recording of the behaviour of the neurites in such cultures was begun on the day following explantation. A fair proportion of cultures then usually showed the beginnings of the outgrowth of neurites, but sometimes they were as yet only to be found in the deeper layers of the plasma clot around the explant; further outgrowth during the course of the day would then bring them near to the undersurface of the cover-slip. Only those neurites at this level were suitable for photography.

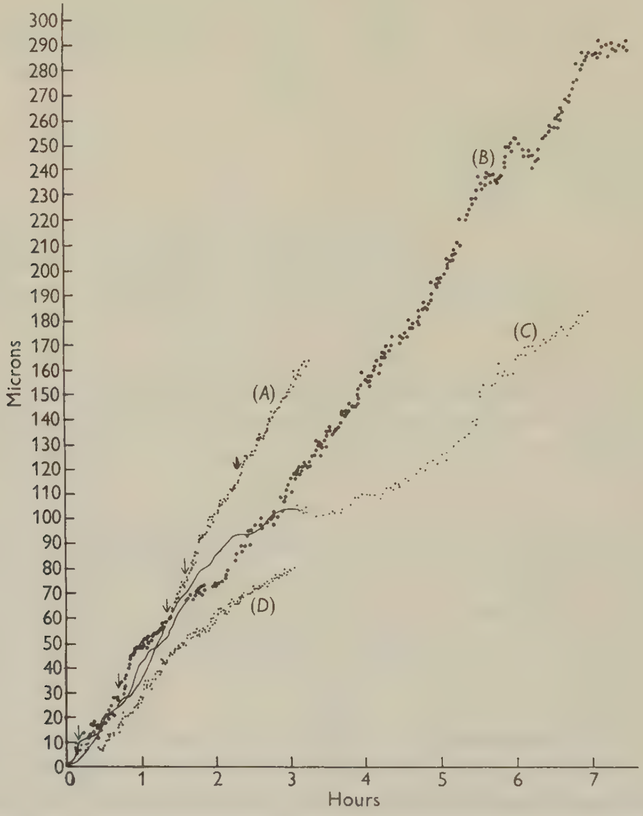
The apparatus used for this purpose, and the technical details, both of the 16 mm. ciné-camera and the photographic methods employed, have already been described (Hughes, 1949), though recent improvements in the mechanism of the camera drive still remain to be published.

The rate of exposure of the film varied between eleven and one frames per minute; the actual speed chosen depended on the magnification used and the rate of movement with the field.

The films after development were analysed by means of a Yade animated viewer, to which was attached a device for counting the frames of the film which was being projected. The magnification on the screen of this instrument was measured and the growth of a neurite was plotted by measuring the distance moved against time, as recorded by the film counter. Whenever a neurite had grown across the field, it was moved back again by means of the mechanical stage of the microscope; these resettings were carefully noted and measured in the analysis of the film. Finally a continuous growth curve was prepared in which these movements were taken into account.

Special features of the technique connected with experiments on the micro-manipulation of cultures will be noticed in the appropriate section below (p. 156). For this work, cultures were mounted in liquid paraffin, in order to prevent evaporation of the culture from the open side of the culture chamber through which the micro-needle is inserted. It was thus necessary to check whether this treatment has any effect on the growth of the culture. No difference could be detected after growth for 48 hr. between normal hanging-drop cultures in contact with air and others in which the air had been replaced by liquid paraffin. In Text-fig. 1 the growth of four neurites over periods of 3–7 hr. is shown graphically. Of these, two (*B* and *C*), were grown in contact with liquid paraffin.

One feature of the technique, however, does affect the growth of the neurite, namely, exposure to light. This sensitivity is generally a limiting factor in time-lapse ciné-micrography of living cells. Even a half-second exposure every minute is sufficient to prevent further growth of a neurite after several hours; the maximum time for which continuous growth was recorded was 7 hours. The rate of elongation is maintained nearly to the moment when growth ceases and the neurite breaks up. Fortunately, there is a clear difference between these periods for which normal growth can be recorded and the duration of survival of the experimentally severed neurites, the observations on which are described in a succeeding section of the paper (p. 156 below, Pl. 1, figs. 2, 3).



Text-fig. 1. The growth of four embryonic neurites in tissue culture, plotted from film records. *A*, the ganglionic neurite of Pl. 1, fig. 1. The arrows point to the occasions, plotted in Text-fig. 2, when vacuoles migrate backwards. *B*, *C* and *D* refer to neurites in cultures of mid-brain. The earlier parts of the curves for *B* and *C* are represented by continuous lines to avoid confusion. *B* and *C* were grown in contact with liquid paraffin.

OBSERVATIONS

(*a*) *General*

The general features of the manner of growth of explants of chick neural tissues have often been described. The earlier literature on the subject is fully reviewed by Levi (1934). Since that time this author has published several further papers in this field, including a definitive study of spinal ganglia in culture (Levi, 1941). The growth of such explants differs from those of fragments of the brain and cord in several ways; the outgrowth consists not only of neurites, but also of fibroblastic cells of neural crest origin, with an extremely high mitotic rate; some of these are closely associated with the neurites in the manner of Schwann cells. Furthermore, after undisturbed growth for a week or so, the central part of the explant has become so thinned by outward migration that the cell bodies of the neurons in this region become accessible to observation (Weiss & Wang, 1936).

The association between neurites and fibroblasts is often very close indeed—much closer than that between a neurite and the surface of a cover-slip against which it is growing. In observing by phase microscopy a neurite growing over a fibroblast in the living state it is often impossible to discern their respective boundaries (Pl. 2, fig. 8). In time-lapse film records, however, one may readily distinguish between them by their distinct and separate types of movement.

Such films convey a striking impression of motion everywhere in the zone of growth. Fibroblasts migrate, and within them is a constant movement of the cytoplasmic inclusions; most neurites are elongating in growth, and exhibit at the tip the activities with which this paper is chiefly concerned.

The cell bodies at the centre of the culture are in complete contrast to this. Their cytoplasmic inclusions, which have a granular appearance under the phase microscope in the living state (Pl. 2, fig. 5), show no detectable movement in a time-lapse film. At the cell surface, however, there are occasionally signs of a rhythmic activity such as Pomerat (1951) has observed in films of explants of adult human brain, though of a much slower periodicity. In these embryonic cell bodies, the interval between successive pulsations is about 20–30 min.

(*b*) *The rate of growth of embryonic neurites*

From a comparison of the data on this subject in the literature certain conclusions may be drawn. The rate of growth is variable and does not remain constant for the individual neurite over periods of more than a few hours. Furthermore, the rate for neurites explanted in tissue culture is apparently higher than for those within the intact embryo.

In his original experiments on the growth of explants of the larval amphibian brain, Harrison (1907) measured rates of growth of 15·6 and 56 μ per hour. One neurite was followed for 53 hr. and grew at an average rate of 21·6 μ per hour. In the tail fin of the intact larvae of several Amphibian species Speidel (1933, p. 6) found that ‘for 5 or 10 minutes a growth cone may extend at an average rate of 1 μ per minute, but this is never maintained. Over a period of 1 hr. an active cone might travel 30–40 μ, while over a 24 hr. period, 200 μ would represent active growth’ (i.e. an average of 8·3 μ per hour).

For the growth of chick neurites in culture, Levi (1934, p. 604) quotes a mean figure of his own of 23·5 μ per hour at 38° C., and a further average of 33 μ per hour at 39° C., due to Mossa (1926), who found that this temperature was the optimal for neurite growth in the chick. The values over periods of 3–7 hr. obtained in this present work vary from 26 to 51 μ per hour (Table 1, Text-fig. 1).

An estimate of the rate of growth of nerve fibres in the intact chick embryo is given by Ramón y Cajal (1928, p. 365). He says that in the 3-day chick it 'approaches 10 μ per hour', though no further details are given.

Table 1. *Data on the growth of neurites in culture*

Source	Culture in contact with	Distance grown in time μ	min.	Rate (μ/min.)
		A. Unoperated neurites		
Lumbar ganglion	Air	162	192	0·85
Mid-brain	Paraffin	295	420	0·70
Mid-brain	Paraffin	148	240	0·62
Mid-brain	Paraffin	182	420	0·43
Mid-brain	Air	80	180	0·45
		B. Cut neurites		
Mid-brain	Paraffin	80	110	0·73
Mid-brain	Paraffin	55	104	0·53
Mid-brain	Paraffin	54	160	0·34
Mid-brain	Paraffin	33	50	0·66
Mid-brain	Paraffin	33	100	0·33
Fore-brain	Paraffin	135	200	0·67
Fore-brain	Paraffin	30	66	0·34
Cervical cord	Paraffin	60	120	0·50

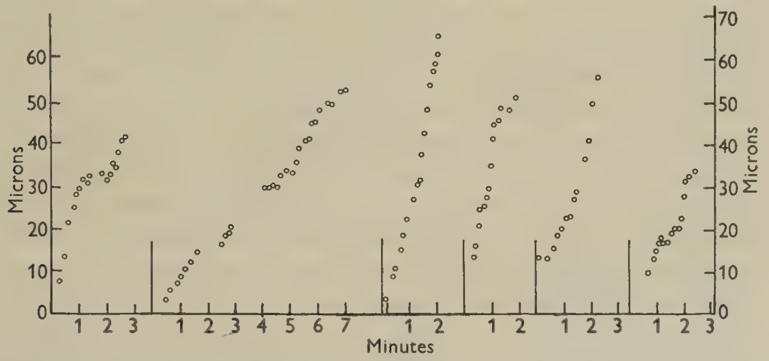
One factor which may be responsible for a slower average rate of growth within the embryo is the inhibitory influence of adjacent neurites upon each other. Neurites growing into the plasma clot of a culture are less crowded together than those within the neural tube of an intact embryo. An example from a culture of a fragment of the mid-brain of the growth of one neurite being hindered by another across its path is given in Pl. 2, fig. 9.

It is noteworthy that even the maximum rate of growth of embryonic neurites is well below the modern estimates of Gutmann, Gutmann, Medawar & Young (1942) for the 'rate of advance of the fastest axon tips' in the regeneration of adult nerves. For branches of the sciatic nerve of the rabbit once the latent period is passed, these authors state that this velocity is from 3·5 to 4·4 mm. per day (146–180 μ per hour).

The comparison of these rates in embryonic and adult tissues will be noticed further on a succeeding page.

(*c*) *Pinocytosis at the tip of the neurite*

In cultures of spinal ganglia in the first and second days after explantation, the early neurites which appear in advance of the fibroblastic outgrowth are easily distinguishable from those in cultures of the brain and cord by reason of their large size, and their complexity of structure at the tip. Fine, uniform regions of neurites from brain and cord explants in solid media are usually about half a micron in diameter; early ganglionic neurites are usually much more irregular in calibre and vary in width from one to several microns. At the tip in healthy examples of both, fine pseudopodial processes in continual movement are extended from the terminal expansion. This structure is very much larger in the ganglionic neurite, and within it numerous vacuoles can often be seen, again in constant movement. The largest of these are about two-thirds of a micron in diameter, while the smallest which can be resolved in these ciné records are rather less than a half of this value. Such vacuoles have already been observed by Matsumoto (1920) in embryonic sympathetic fibres *in vitro*, and by Lewis (1950) in ganglionic neurites. A further feature of the tip of ganglionic neurites which Lewis also described, is an undulating membrane, comparable to that of a macrophage, which like that of other cells, can enfold vacuoles of external fluid by the process which was originally described by the same author as 'pinocytosis'. (Lewis, 1931).



Text-fig. 2. Migration of vacuoles towards cell-body in the ganglionic neurite of Text-fig. 1*A* and Pl. 1, fig. 1. Distances are measured from the neurite tip.

Figs. 6*a*, *b* and 8*a* of Pl. 2 of this paper illustrate the terminal undulatory membrane in a spinal neurite. It appears to form a web stretched between filamentous pseudopods, though these may possibly be folds in a continuous structure.

It is very probable that the entry of vacuoles into the tip of the neurite is thus a consequence of its protoplasmic activity. New vacuoles are formed almost continuously at the tip of an active neurite, though not always is a continuous undulatory membrane visible; filamentous pseudopods also seem to bring vacuoles into the neurite tip.

What, therefore, is the fate of these vacuoles which accumulate within the neuroplasm? Near the tip they seem mostly to move at random along the neurite but occasionally one is seen in the film records to move rapidly and continuously away from the tip, and towards the cell body. In one particular record of the behaviour of a ganglionic neurite over a period of 3 hr., this phenomenon was studied in some detail (Text-fig. 2). The limitation on the recording of the movement of one particular object of the size of such a vacuole is the extremely small depth of focus of the phase microscope at high powers. Even within a neurite closely applied to the underside of the cover-slip, a vacuole can readily move in and out of the plane of focus. However, six clear instances of the continuous movement of vacuoles in the direction of the cell body over distances of 12–28 μ were found in this record. Their velocities varied from 3·8 to 17 μ per minute.

Careful search has revealed examples of this centripetal movement in most of the records of the behaviour of neurites which have been obtained during the course of this work.

It cannot yet be decided whether periods of flow in each direction along the neurite alternate with each other, or whether streams of 'raw material' taken in at the tip, and of formed protoplasm proceeding from the cell body, can simultaneously flow in opposite ways along so small a structure as the embryonic nerve fibre.

(*d*) *Surface behaviour in other types of cell in neural explants*

Macrophages become increasingly evident as the age of all such cultures advances, and finally are still active when all other cells are moribund. Macrophages of a flattened epithelioid type are conspicuous in cultures of spinal cord grown in fluid medium in the first few days after explantation. In them, the process of pinocytosis is especially clear; a series of stages in the formation of a vacuole is shown in Pl. 2, fig. 4. Comparison of this figure with those of ganglionic neurites shows how much larger are the vacuoles in macrophages.

From the edge of an explant of spinal cord there often grows outward a coherent epithelium of supporting elements, the marginal cells of which have undulating membranes (Pl. 2, fig. 7), due to the activity of which small vacuoles are continuously entering this epithelium. It is not clear whether embryonic sustentacular cells normally behave in this way, or whether their pinocytic activity is here merely a response to the conditions of tissue culture.

(*e*) *The effect of severing a neurite from contact with its cell body*

The main paper in the literature on the micro-manipulation of cultures of nervous tissues is that of Levi & Meyer (1945). These authors summarize the results of previous studies of this kind. In 1926 Levi showed that if the nucleus of a neuroblast was pierced with a micro-needle, the cell body degenerated rapidly, but that there was an interval of time before the autolysis of its neurites. Levi & Meyer (1945) made cuts with a micro-needle through a plexus of neurites in the outgrowth of spinal ganglion cultures, and found that healing could take place within a few hours without degeneration of the region of the plexus distal to the cut.

The object of the present experiments was to sever a neurite near its emergence from the explant, and to follow the subsequent fate of the distal portion. A simple micro-manipulator which gave vertical and transverse motion in one other plane was constructed, and attempts were made to sever neurites in culture by means of a needle formed by honing a piece of 0·2 mm. stainless steel wire to a pointed tip a few microns across. However, it was found that the plasma coagulum could only be torn after a degree of disturbance which rendered the operated region of the culture unfit for further high-power observation. Fortunately, a way out of this difficulty was provided by the area of liquefaction of the clot which usually develops around explants of the brain and cord, which is due, as Weiss (1934) has shown, to the proteolytic activity of the ependymal layer. The neurites in this zone are slightly stretched across a fluid zone between explant and the unliquefied clot beyond, in which the neurites are still growing (Pl. 1, figs. 2*a*, 3*a*). It is comparatively easy to tear away neurites from this proximal zone, and to leave undisturbed their distal extremities.

When this operation is performed it is found that these neurites deprived of connexion with their cell bodies do not immediately die, but continue to grow for periods of one or more hours. Around cultures of spinal ganglia, the medium does not liquefy; both for this reason and because of the presence of Schwann fibroblasts in these cultures, it was decided to use only explants of the brain and cord for these particular experiments, although their neurites are less spectacular than those of spinal ganglia.

Explants of fragments of the brain and cord at 7–8 days were found most satisfactory for this type of experiment. From each batch of cultures it was necessary to choose examples in which the neurites were in an early stage of outgrowth, and where, next to the explant, there was a suitable zone of liquefaction of the medium from 100–200 μ in width. The $\frac{7}{8}$ in. square cover-slip bearing a suitable culture was mounted in ordinary medicinal liquid paraffin over a glass cell, which gave support on three sides, leaving the fourth open for the introduction of the micro-needle. In this way, drying-up of the culture during the experiment is avoided; this element of the technique is of course due to de Fonbrune who uses it for his exquisite micro-manipulator. No difference could be detected in the growth of cultures in contact with liquid paraffin and those sealed over air in the ordinary way.

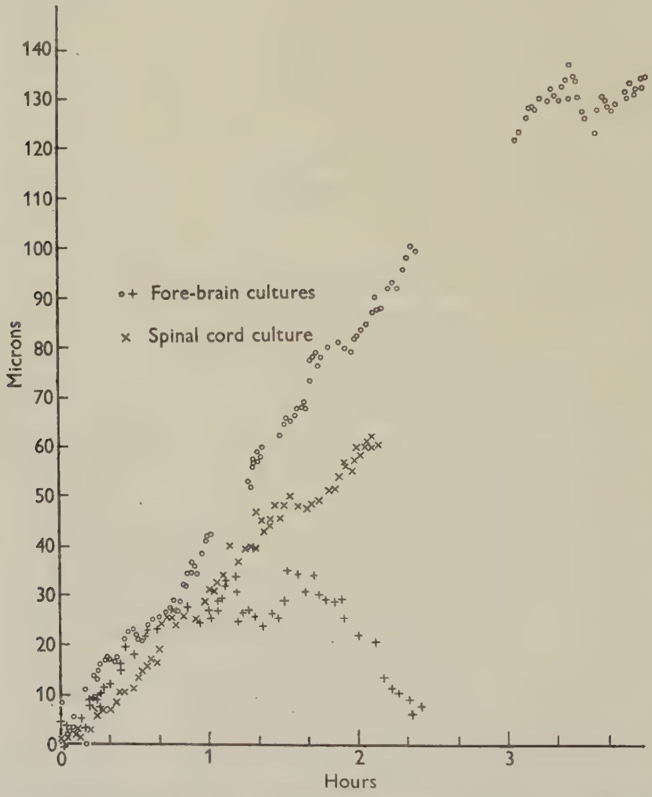
The neurites were torn away from the zone of liquefaction under a × 10 objective, the field of operation being photographed before and after the act. At higher magnification, the severed neurites in the plasma beyond were then inspected, and one was chosen in which there could be no doubt that contact with the cell body had been interrupted. Glial cells, together with a few neuroblasts, were usually to be found on the far side of the zone of operation, and so careful scrutiny was necessary to ensure that the neurite chosen for observation did not proceed from a neuroblast drawn away from the main body of cells in the explant. Sometimes it was necessary to operate on several cultures in succession before a suitable neurite was found.

In different experiments, both ends of the isolated neurite were studied. The proximal end, where the break has been made, becomes club-shaped (Pl. 1, fig. 2*c*, *d*); vacuoles collect within it, and a film record of its behaviour shows that at the surface there is continual movement during its period of survival. During this time growth continues at the tip of the isolated neurite. Eight records were made of the growth of severed neurites; they advanced for distances which varied between 33 and 135 microns at rates of 0·33 to 0·73 μ per minute (Table 1, Text-fig. 3). This range of velocity suggests no difference from that observed in unoperated cultures.

Growth ceased in the operated neurites at periods of 50–200 min. after operation; fine pseudopodia were then no longer emitted from the tip, which usually began to retract. The outline of the whole became irregular, and sooner or later broke up into round protoplasmic drops (Pl. 1, fig. 3*c*).

DISCUSSION

The analogy of the tip of a living embryonic neurite with an amoeba has suggested a general picture of its movement and extension, although it may be doubted whether a complete description of the processes has yet been given. Usually (Weiss, 1941; Lewis, 1945, 1950) it is stated that fine pseudopodia are extended from the nerve tip, most of which are later withdrawn; endoplasm, however, flows into one or perhaps two of these, and so the neurite advances by the enlargement of a pseudopodium into a new section of the neurite. Thus Weiss (1941, p. 175): 'once a pseudopodium has established itself and caused the mass of protoplasm to flow into it, the drain thereby exerted on the other pseudopodia produces their automatic withdrawal.' Lewis stresses the role played by the outer layer of contractile gel over the neurite, which forces endoplasm into the persisting process. He says: 'The weakest part—the weak gel layer of the terminal pseudopod—is expanded and advanced', though this inference, as he admits, is mainly based on the study of the locomotion of amoebae.



Text-fig. 3. Growth of three neurites after severing connexion with the cell body in the explant. Data on further experiments of this kind is given in Table 1.

Close observation of the film records of the growth of the neurites in spinal ganglion cultures in this present work has led me to question this view. In actual fact the rapidity with which the pattern of extrusion of the fine pseudopodia changes is far too great to allow for the persistence of any individual one of them. The main tip of the neurite advances discontinuously, in separate thrusts; during one of these forward movements several completely fresh sets of fine pseudopodia will be extruded and withdrawn. It is thus impossible for the terminal pseudopodia to serve as the means by which the neurite is anchored to its surroundings.

Again, the distal flow of endoplasm through the neurite is regarded as the immediate cause of the forward growth of the neurite (Weiss, 1944). In post-embryonic nerves, Weiss & Hiscoe (1948) have assembled an impressive body of experimental evidence on this question, mainly relating to the damming of axoplasm when a nerve is constricted along its course. They conclude that 'growth in the sense of production of new protoplasm occurs solely at the base of the fiber in the nucleated part of the cell body'.

For the embryonic neurite, however, the results of the isolation experiments described in this paper show that growth in the embryonic neurite does not immediately depend upon protoplasmic synthesis of this kind. The distal part of a neurite continues to grow at the normal rate for a period of one to several hours after its connexion with the cell body has been destroyed.

To what, then, is the growth of an embryonic neurite immediately due? The probable cause is the intake of fluid. In the large neurites of spinal ganglion cultures, and in those of the brain and cord grown in fluid media, the process is microscopically visible as terminal pinocytosis. In smaller neurites, where this process cannot be observed at the tip, it is probable that water is also being absorbed, though on a smaller scale, for visible vacuoles have been seen to collect at the proximal end of a neurite isolated from its cell body in a culture of mid-brain in a plasma clot (Pl. 1, fig. 2*c*, *d*).

After several hours' growth of a severed neurite it stains noticeably paler with haematoxylin than the unoperated neurites in the same culture. This observation suggests a comparison with the recent work of Brachet on halved amoebae (Brachet, 1952). This author divided *Amoeba proteus* into two portions, one of which contained the nucleus. Both halves were starved, and after 10 days the main difference between them was that the basophilia of the cytoplasm of that without a nucleus rapidly decreased. It would be of great interest to know whether Brachet's inference from this work, concerning the function of the nucleus in coupling oxidation and phosphorylation, is also true of the neuroblast.

One function, if not the main one, of the terminal pseudopodia of the neurite is to bring in water and dissolved substances by pinocytosis. The details of the process are not yet fully understood, but the analogy with the activity of the undulating membrane of macrophages is clear. In them, the membrane seems first to fold round and enclose a portion of the external fluid (Pl. 2, fig. 4). This passes in towards the main body of the cell, and it is not until then that the new vacuole becomes rounded and spherical by surface-tension forces; its area in the photomicrographs has then markedly decreased. It thus may well be that over the surface of the undulating membrance there is some surface-active substance such as has been proved to exist at the surface of marine eggs (Danielli, 1945).

At any rate, it is true to say that where no processes are being emitted at the tip of neurites, then there is no increase in length. There is, however, no uniform relationship between the rate at which a neurite advances, and the degree of pseudopodial activity at the tip. In fluid media, the latter is very marked, and yet the velocity of progression of the neurite may be low. This difference between growth in solid, and fluid media is particularly striking in the neurites from explants of the brain and cord.

The actual rate at which a neurite is growing must depend upon a number of factors: the intake of water at the tip and its passage in vacuoles towards the cell body; the contrary flow of synthetized protoplasm, the almost peristaltic movement of the neurite due to its contractile gel layer; and the tactile influences exerted on the neurite tip by its surroundings, must all play their several parts in the growth of the embryonic nerve fibre.

It may well be that there is a real difference in the growth of embryonic and adult nerve fibres in the degree to which elongation is immediately dependent upon the flow of axoplasm towards the advancing tip, and that this distinction is related to the difference in their respective rates of growth. It would be of great value if the regeneration of an individual nerve fibre could be observed in the adult higher animal.

SUMMARY

1. The growth of neurites in cultures of explants of chick neural tissues has been observed by means of phase-contrast ciné-microscopy. The rate of growth is of the same order which has been measured by previous authors.
2. The activity of the tip of the neurite has been studied. The intake of vacuoles of external fluid has been confirmed, and it is concluded that this is the main function of the movement of the terminal pseudopodia and undulating membranes.
3. Vacuoles at the tip have been seen to pass at intervals down the neurite, towards the cell body.
4. Cutting of the neurite close to the explant does not immediately lead to the death of the severed distal portion, which continues to grow for periods of 1–3 hr.
5. It is concluded that the immediate cause of the growth of embryonic neurites is the intake of water at the tip.
6. The suggestion is made that regenerating axons of the adult animal are more closely dependent on the flow of synthesized protoplasm from the cell body and that this postulated difference is correlated with their more rapid rate of growth.

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REFERENCES

Brachet, J. (1952). The role of the nucleus and the cytoplasm in synthesis and morphogenesis. *Symp. Soc. exp. Biol.* no. vi, pp. 173–200.

Burrows, M. T. (1911). The growth of tissues of the chick embryo outside the animal body, with special reference to the nervous system. *J. exp. Zool.* **10**, 63–84.

Costero, I. & Pomerat, C. M. (1951). Cultivation of neurons from the adult human cerebral and cerebellar cortex. *Amer. J. Anat.* **89**, 405–467.

Danielli, J. F. (1945). Some reflections on the forms of simpler cells. *Essays on Growth and Form.* Ed. by W. E. Le Gros Clark & P. B. Medawar, pp. 295–308. Oxford: Clarendon Press.

Gutmann, E., Gutmann, L., Medawar, P. B. & Young, J. Z. (1942). The rate of regeneration of nerve. *J. exp. Biol.* **19**, 14–44.

Harrison, R. G. (1907). Observations on the living developing nerve fiber. *Anat. Rec.* **1**, 116–118.

Harrison, R. G. (1910). The outgrowth of the nerve fiber as a mode of protoplasmic movement *J. exp. Zool.* **9**, 787–848.

Hughes, A. F. W. (1949). The technique of kine-photomicrography of living cells. *J. R. micr. Soc.* **69**, 53–64.

Levi, G. (1917). Connessioni e struttura degli elementi nervosi sviluppati fuori dell'organismo. *Mem. Accad. Lincei*, **12**, 142–182.

Levi, G. (1926). Ricerche sperimentali sovra elementi nervosi sviluppati 'in vitro'. *Arch. Exp. Zellforsch.* **2**, 244–272.

Levi, G. (1934). Explantation, besonders die Struktur und die biologischen Eigenschaften der in vitro gezüchteten Zellen und Gewebe. *Z. ges. Anat.* 1. *Ergebn. Anat. EntwGesch.* **31**, 125–707.

Levi, G. (1941). Nouvelles recherches sur le tissu nerveux cultivé in vitro; morphologie, croissance et relations réciproques des neurons. *Arch. de Biol.* **52**, 133–278.

Levi, G. & Meyer, H. (1945). Reactive, regressive and regenerative processes of neurons, cultivated in vitro and injured with the micromanipulator. *J. exp. Zool.* **99**, 141–181.

Lewis, W. H. (1931). Pinocytosis. *Johns Hopk. Hosp. Bull.* **49**, 17–26.

Lewis, W. H. (1945). Axon growth and regeneration. *Anat. Rec.* **91**, 287.

Lewis, W. H. (1950). Motion picture of neurons and neuroglia in tissue culture. In *Genetic Neurology*. Ed. by P. Weiss. Chicago: University of Chicago Press.

Lewis, W. H. & Lewis, Margaret R. (1912). The cultivation of sympathetic nerves from the intestine of chick embryos in saline solutions. *Anat. Rec.* **6**, 7–31.

Matsumoto, T. (1920). The granules, vacuoles and mitochondria in the sympathetic nerve-fibers cultivated *in vitro*. *Johns Hopk. Hosp. Bull.* **31**, 91–93.

Mossa, S. (1926). La vitesse d'accroisement des fibres nerveuses cultivées *in vitro* en function de la température. *C.R. Ass. Anat.* **21**, 403–407.

Pomerat, C. M. (1951). Pulsatile activity of cells from the human brain in tissue culture. *J. nerv. ment. Dis.* **114**, 430–449.

Ramón y Cajal, S. (1890). A quelle époque apparaissent les expansions des cellules nerveuses de la moëlle épinière du poulet. *Anat. Anz.* **5**, 609–613, 631–639.

Ramón y Cajal, S. (1928). *Degeneration and Regeneration of the Nervous System.* Trans. R. M. May. Oxford: University Press.

Speidel, C. C. (1932). Studies of living nerves. I. The movements of individual sheath cells and nerve sprouts correlated with the process of myelin-sheath formation in amphibian larvae. *J. exp. Zool.* **61**, 279–331.

Speidel, C. C. (1933). Studies of living nerves. II. Activities of ameboid growth cones, sheath cells and myelin segments, as revealed by prolonged observation of individual nerve fibers in frog tadpoles. *Amer. J. Anat.* **52**, 1–79.

Weiss, P. (1934). Secretory activity of the inner layer of the embryonic mid-brain in the chick, as revealed by tissue culture. *Anat. Rec.* **58**, 299–302.

Weiss, P. (1941). Nerve patterns. The mechanics of nerve growth. *3rd Growth Symposium, Growth*, **5** (supp.), 163–203.

Weiss, P. (1944). Damming of axoplasm in constricted nerve, a sign of perpetual growth in nerve fibres. *Anat. Rec.* **88**, 464.

Weiss, P. & Hiscoe, H. B. (1948). Experiments on the mechanism of nerve growth. *J. exp. Zool.* **107**, 315–395.

Weiss, P. & Wang, H. (1936). Neurofibrils in living ganglion cells of the chick, cultivated *in vitro*. *Anat. Rec.* **67**, 105–117.

EXPLANATION OF PLATES

All figures are of phase-contrast photomicrographs of living cells, or parts of cells, from explants of the nervous system of chick embryos, grown in tissue culture. They are enlargements from single frames of 16 mm. time-lapse sequences. Figs. 2*a*, *b* and 3*a*, *b* are at low-power; for the remainder a 2 mm. immersion lens was used, and they are to a magnification of × 1400.

Plate 1

Fig. 1*a*, *b*. Tip of neurite from a culture of a lumbar dorsal root ganglion of a 7-day chick, cultivated for 2 days. *a–d* and *e–h* are sequences separated by an interval of just over 1 hr. They each show the passage of a vacuole down the neurite in the direction of the cell body. (*b*), 1·1 min., (*c*), 3 min., (*d*), 5 min. after (*a*), (*f*) 0·8 min., (*g*) 1·0 min., (*h*) 1·2 min. after (*e*).

Fig. 2*a–d*. An experiment in which neurites were severed in a 24 hr. culture of 6–day optic lobe. (*a*) Part of the culture at low-power, showing the proximal zone of neurites in the zone of liquefied medium. (*b*) Same field 8 min. later after the neurites have been removed from this region. (*c*) Proximal end of one isolated neurite in this field under high-power, 13 min. after operation. It becomes club-shaped and vacuoles collected within. (*d*) Same field 204 min. after operation. The neurite still shows movement.

Fig. 3*a–e*. Observation on the tip of a neurite in a similar experiment. (*a*) Field under low-power before operation, showing neurites in zone of liquefaction adjacent to explant. (*b*) Same field 3 min. later, showing neurites removed from this zone. (*c*) Tip of one severed neurite in plasma clot beyond, under high-power; 9 min. after operation. (*d*) Same tip 98 min. after operation, after having grown outwards for a further 72μ. (*e*) Same tip 132 min. after operation, now degenerating.

PLATE 2

Fig. 4*a–d*. A macrophage from a culture of 6–day spinal cord in fluid medium, during the third day after explantation, to show the intake by pinocytosis of a vacuole indicated by an arrow. (*b*) 1·6 sec., (*c*) 7·5 sec., (*d*) 14·4 sec. after (*a*).

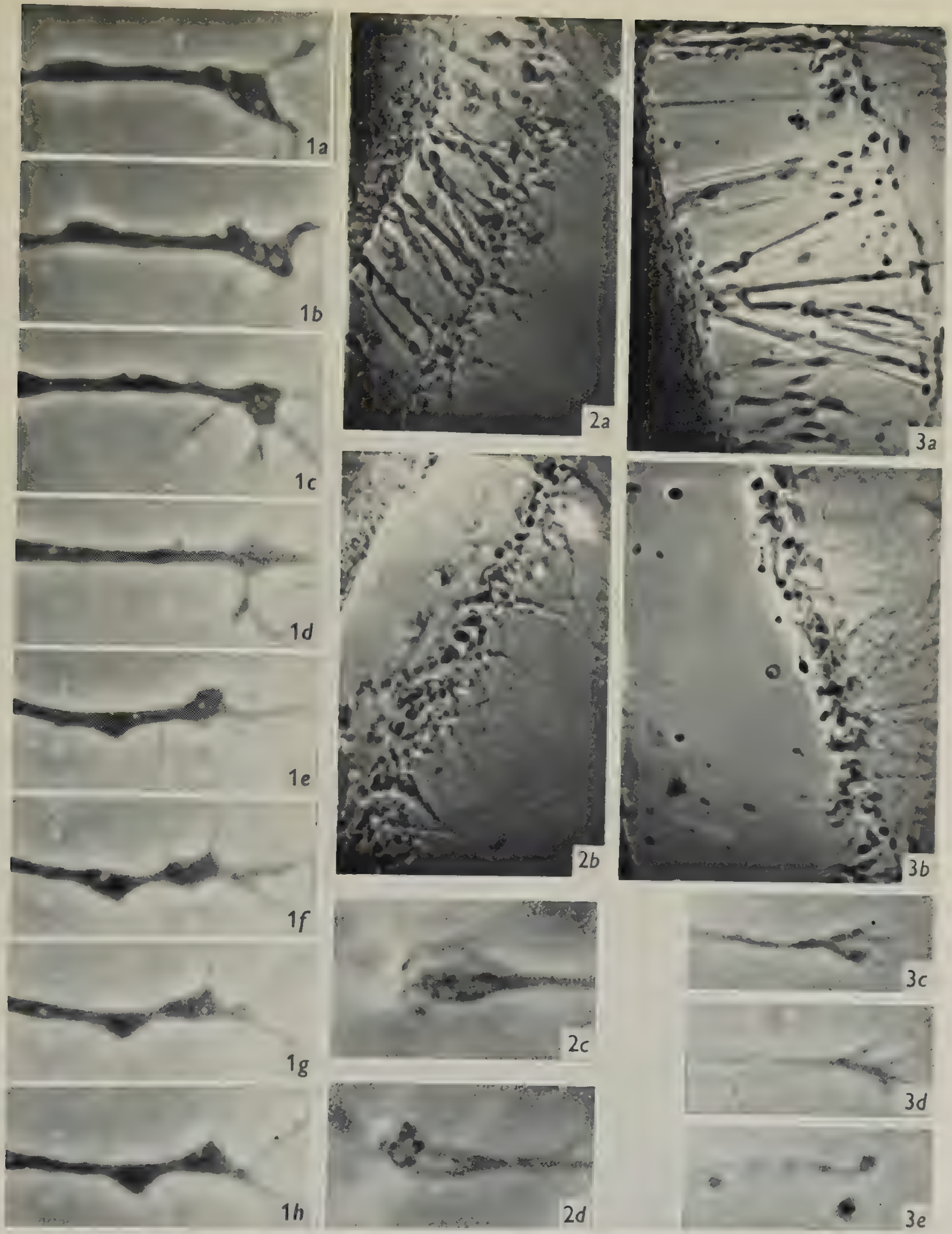
Fig. 5. Cell body in 3–day culture of 9–day lumbar spinal ganglion, to show neurofibrillae in perinuclear cytoplasm. They are granular in appearance by phase-contrast.

Fig. 6*a, b*. Ganglionic neurite in the same culture on the previous day, to show a terminal undulating membrane. (*a*) and (*b*) are 6 min. apart.

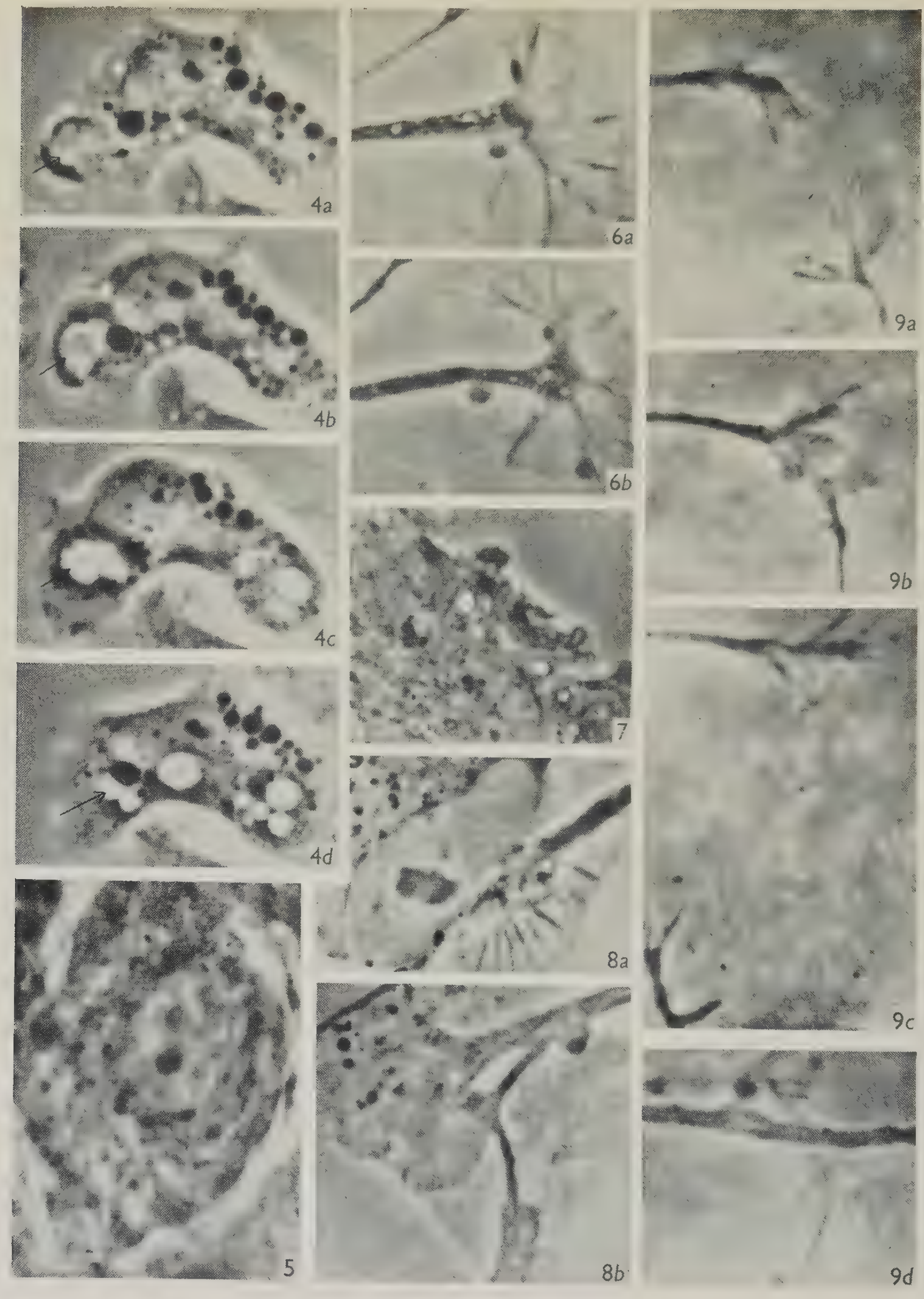
Fig. 7. The outgrowth from an explant of 9-day spinal cord grown in fluid medium for three days. Supporting elements have formed an epithelium, at the margin of which cells are actively pinocytic.

Fig. 8. The overgrowth of a Schwann fibroblast by a ganglionic neurite in the same culture as in figs. 5 and 6. (*a*) The neurite tip with undulating membrane is to the right of the cell nucleus. (*b*) 37 min. later. The neurite has covered the nucleus and much of the cytoplasm of the fibroblast.

Fig. 9. Part of the outgrowth of a 24 hr. culture of a fragment of 6-day optic lobe, to show the interaction of two neurites, which approached each other at right angles (*a*). (*b*) 9·4 min. later than (*a*); the two tips have met. (*c*) 50 min. later than (*a*); the first has retreated, while the second continues to grow. (*d*) 115 min. later than (*a*); the first has now grown over the second, the tip of which by now is approximately 50μ further than in (*a*).



HUGHES—GROWTH OF EMBRYONIC NEURITES



HUGHES—GROWTH OF EMBRYONIC NEURITES

PATTERNS OF CUTANEOUS PIGMENTATION: RODENTS

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INTRODUCTION

One of the foremost discoveries in the field of pigmentation was the discovery of the Dopa reagent, and its subsequent application to normal and pathological pigmented tissues (Becker, 1927; Becker, Praver & Thatcher, 1935; Bloch, 1929). This reagent enables the histologist to differentiate between those cells which form pigment (melanoblasts) and those which acquire it secondarily (melanophores).

Bloch (1929) distinguished two types of melanoblast: those associated with the epidermis, which he thought developed by metaplasia of epidermal cells, and those of the dermis which he regarded as mesodermal elements. To the latter he gave the name Mongolen-Zellen because they were found in the Mongolian spots of human newborn. He drew attention to the fact that in some species (e.g. Japanese negro fowl) mesodermal pigmentation is predominant.

The branching form of many pigment-containing cells has long been described but the term *dentritic cells*, although not of recent origin, is only now coming into general usage. The study of the relationship of these cells to the epidermis has been greatly facilitated by the use of tryptic digestion to obtain pure sheets of epidermis isolated from the dermis (Medawar, 1941). By means of this method, together with older routine histological techniques, Billingham (1948, 1949) has been able to present a clear picture of the pigmentary systems in guinea-pig and human skin.

Epidermal pigment occurs in two types of cells: (1) the dendritic cells mentioned above, which lie along the epidermodermal junction with processes extending up between the basal epidermal cells; and (2) in the epidermal cells themselves. Only the dendritic cells are Dopa positive, and they alone form pigment. The epidermal cells acquire their melanin secondarily from the dendritic cells, probably by a process of inoculation. This latter mechanism was noted in Amphibia by Stearner (1946), and Billingham (1948) describes 'end-feet' of dendritic cell processes closely applied to epidermal cells.

A system of non-pigmented or 'white' dendritic cells has been described by Billingham & Medawar (1947) and Billingham (1948, 1949) in non-pigmented skin of spotted guinea-pigs, and in human skin. These resemble the pigmentary dentritic cells in all respects except that of melanogenesis.

Pigment is also found in the dermis. Bloch's 'mesodermal melanoblasts' have already been referred to, and Steiner-Wourlisch (1925) described a dermal dendritic cell system in the mouse. Becker (1927) regarded dermal pigment as derived from the epidermis, being phagocytosed by dermal cells. Miescher (1922) injected melanin into pigment-free skin, and found that the melanin was ingested by connective tissue cells which could not then be distinguished from natural melanophores.

Hair follicles giving rise to pigmented hairs also show dendritic cells, and Chase, Rauch & Smith (1951) noted their appearance in the follicles of developing mouse hair. Chase & Smith (1949) commented that contact between matrix cells and dendritic processes was necessary for pigmentation of the former cells.

A COMPARATIVE STUDY OF PIGMENTATION IN FOUR RODENTS

Material and methods

The skin pigmentation of the rat, mouse, guinea-pig and rabbit has been studied. Methods used have included paraffin sections stained by various techniques, the use of the 'split-skin' technique of Medawar, and the application of the Dopa reagent, both to tissues in bulk (Becker, Praver & Thatcher, 1935), to frozen sections, and to split skin preparations (Pl. 1, fig. 1). Ear skin as well as body skin has been examined.

In the present study the pattern of pigmentation in the mouse and rat was found to agree with that described by Steiner-Wourlisch (1925) in the mouse. Briefly, rat and mouse ear skin shows a preponderance of dermal pigment (Pl. 1, fig. 2), with dendritic and non-dendritic cell types lying within the upper dermis. Dendritic cells are also seen in contact with the basal epidermal cells between which their processes extend. Pigment granules are also found within the epidermal cells, but distribution over the ear is not uniform.

Body skin of the two rodents shows no pigment cells in either the dermis or the epidermis. Dendritic cells, however, occur in the hair follicles of pigmented hairs, where they lie amongst the cells of the hair matrix, and also at the junction between the matrix and the dermal papilla.

The pigmentary system of guinea-pig skin was described by Billingham (1948). Comparison of guinea-pig ear skin with that of the rat and mouse shows a shift in pigmentation towards the epidermis in the guinea-pig (Pl. 1, fig. 3). A dense dendritic cell system lies at the dermo-epidermal junction, and many processes from these cells extend between the basal epidermal cells. Heavy pigment deposits are found within epidermal cells. Dermal pigment is present, but is never so marked as in the rat and mouse and dermal dendritic cells are few. Most of the dermal pigment lies deep to the denser patches of epidermal pigment, and hence deep to the areas of greatest density of dendritic cells along the dermo-epidermal junction.

Body skin of the guinea-pig differs from that of the rat and mouse in that dendritic cells are present along the epidermo-dermal region (Pl. 1, fig. 5), as in the ear, but in less density. Pigment is present in the epidermal cells here; dermal pigment is minimal, and dermal dendritic cells have not been seen. As in other rodents, pigment is found in the hair matrices both in the matrix cells and in dendritic cells.

In the black rabbit ear-skin pigmentation is generally slighter, and almost entirely confined to the epidermis (Pl. 1, fig. 4). Dendritic cells occur in the usual situation at the epidermo-dermal junction and rarely in the extreme upper dermis. Dermal pigment, however, is almost completely absent. The body skin of the rabbit has no dendritic cells or pigment, except within the hair follicles.

Rodent skin thus shows three types of pigment-bearing cells. Pigmentary dendritic cells lie at the epidermo-dermal junction and among the basal epidermal cells. They occur frequently within the dermis of the rat and mouse, less so in that of the guinea-pig, and rarely in the rabbit. This cell is always Dopa-positive, and is the only cell to synthetize melanin. It is thus inseparable from pigmented areas.

The other two types of pigment-containing cells are the epidermal cell itself, and the reputedly phagocytic dermal melanophore. Hair follicles contain pigment both in the matrix cells, which are epidermal in origin, and also in dendritic cells.

Phagocytosis by dermal melanophores

Dermal pigment cells have long been considered to have phagocytic properties, and this has been demonstrated experimentally by Miescher (1922). In view of the intense dermal pigmentation observed in the present study in certain of the rodents used, experiments were performed to investigate the phagocytic properties of dermal melanophores.

Subcutaneous injections of 1 % trypan blue were made in piebald guinea-pigs and mice. Three mice and two guinea-pigs received 2–3 g./kg. of body weight over 6–7 days. Whole thickness pieces of skin were then fixed in 10 % formol, and paraffin sections made. Ear skin showed numerous phagocytic cells containing blue granules lying throughout the dermis, and some of these cells also contained granules of melanin. Certain melanotic cells which were densely packed with pigment showed no blue granules; these cells may have been filled to capacity with pigment, or it is possible that the melanin may have masked the blue dye. Dendritic cells and basal epidermal cells in no instance showed any phagocytosis.

In the deeper parts of the dermis many cells were found with heavy concentrations of blue pigment, but in none of these was it possible to detect melanin.

Intracellular disposition of pigment

Examination of pigmented epidermis shows a definite localization of pigment within the epidermal cells. This consists of a concentration of pigment over the superficial pole of the nucleus, i.e. 'nuclear capping' (Pl. 1, figs. 3, 4). This disposition has been noted by various observers, including Ludford (1924) in the horse, and Billingham (1948, 1949) in guinea-pig and human skin.

The rodent skin examined in this study shows obvious nuclear capping wherever pigmented epidermal cells occur. Sections of macaque monkey and human skin have also been examined, and here capping is obvious. Its presence is irrespective of the hair density over the skin in question, although it is more obvious where most pigment is present.

Nuclear capping and hair pigmentation

Examination of the hair of rodents, either in sections of skin, or simply cleared and mounted in balsam, shows well-defined cortical and medullary components. In contrast to human hair, where the pigment is predominantly cortical, the rodents show obvious medullary pigmentation (Pl. 1, figs. 6, 7). Hausman (1928) draws attention to the fact that a variety of pigment characters may be seen within a single hair, varying from root to tip.

Medullary pigmentation is especially obvious in the mouse and rat, and here cortical pigment is slight. The medullary pigment lies in transverse bands, separated by pigment-free and apparently empty spaces. The bands are made up of close-packed melanin granules. Cortical pigment consists of linear groups of granules, extending lengthwise along the hair.

Examination of the basal region of the follicle shows that the clear spaces of the medulla are initially occupied by nuclei (Pl. 1, fig. 8), which break up and disappear towards the distal part of the hair. The banding of melanin appears originally as a nuclear capping in the matrix cells giving rise to the hair medulla. As these cells come to lie along the axis of the medulla, the pigment of one cell is separated from that of the next distal by the pigment-free part of the latter's body, containing the nucleus. Disappearance of the nuclei will thus leave the pigment in bands, separated by the spaces originally occupied by nuclei (Pl. 1, fig. 6).

DISCUSSION

The four rodents studied show marked differences in their patterns of pigmentation. Ear and body skin from any one animal shows a characteristic histological picture, as well as having obvious gross differences in pigmentation and hair density.

In ear skin there is a shift in the location of pigment from a predominantly dermal distribution in the mouse and rat to a predominantly epidermal one in the rabbit; the guinea-pig occupies an intermediate position. Dense dermal pigmentation is associated with dendritic cells lying actually within the dermis. In the guinea-pig these are fewer in number, but there is a dense system of dendritic cells at the epidermo-dermal junction. The rabbit, with fewer basal dendritic cells and hardly any within the dermis, shows a minimal dermal pigmentation.

The guinea-pig is the only one of the four to show pigmentary dendritic cells in relation to the epidermis over the body. Slight dermal pigmentation is also found in this animal, but not in the other three.

On the basis of these observations it is clear that melanin does not occur in the absence of pigmentary dendritic cells. They have been found at all sites of pigment deposition in these rodents. Pigment density in any epidermal or dermal area decreases as the density of the dendritic cell system in relation to that area diminishes. This has so far been shown to hold only with regard to those dendritic cells giving a positive Dopa reaction.

Intracellular disposition of pigment

Two kinds of Dopa-negative pigment-containing cells have been described. One of these, shown to be possessed of phagocytic properties, lies in the dermis. Cells of this type show no precise intracellular localization of pigment. The second type, however, is the pigmented epidermal cell which is non-phagocytic and probably acquires its pigment by a process of direct inoculation; such cells commonly show nuclear capping of pigment. This latter type of localization is commonly attributed to the function of the pigment in shielding the nucleus from the harmful effects of undue ultraviolet radiation. This is not a direct cause-effect relationship, however, for capping is seen in very young animals, in cells covered by a thick coat of hair, and in epidermal derivatives well below epidermal level, as in hair matrices. It is widely seen throughout mammals, and appears to be a definite characteristic of epidermal cells.

No such capping is seen in dermal melanophores, as mentioned above, whatever their position may be within the dermis. Many of these lie directly subepidermally, considerably nearer to the skin surface than those epidermal cells making up the deeper parts of the hair follicles.

The mechanism of production of the prominent transverse striations of hair was referred to earlier. It is noteworthy that this is seen only in the medulla, where the cells, although degenerate, maintain a spatial relationship to each other similar to that existing in the matrix itself. In the hair cortex, however, the cells become elongated and compressed and it is not possible to detect any persistence of nuclear capping.

Dermal and epidermal dendritic cells

The dentritic cells have been shown to develop from neural crest elements in Amphibia, birds, and more recently in the mouse (Rawles, 1947). There is no reason to suppose that the dermal and epidermal dendritic cells do not have the same origin, as they are very similar in form and function in the two situations.

On one point, however, some doubt remains. It is uncertain whether dendritic cells lying in the dermis have end-feet applied to dermal melanophores. Dopa-treated sections show terminal expansions of dendritic processes, apparently similar to the end-feet applied to epidermal cells; but it is difficult to say whether these are in contact with dermal phagocytic cells or not. Billingham (1948) described end-feet from one cell ending on the cell body of another, and this may be the explanation of the terminal expansions seen.

Pigment acquisition by dermal melanophores

The phagocytic properties of these cells have already been referred to. Whether this is their only means of acquiring pigment is not known. If the end-feet mentioned above are actually applied to melanophores, presumably direct injection could take place. It seems that these phagocytic cells must lie close to the pigment-producing cells in order to acquire pigment; deep dermal phagocytes in mouse and guinea-pig skin showed no pigment, while the more superficial ones, lying close to the dendritic cells, were packed with pigment granules. Where dense deposits of pigment are separated from phagocytes by the presence of non-pigmented epidermis, the phagocytes remain non-pigmented. This is seen in hair follicles where pigment is very commonly found in the matrix. This pigment does not, however, pass out to the adjacent dermis owing to the interposition of non-pigmented epidermal cells at the periphery of the follicle.

SUMMARY

1. Cutaneous pigment in the rat, mouse, guinea-pig and rabbit shows epidermal and dermal components. In pigmented ear skin, dermal pigmentation is heavy in the rat and mouse, less so in the guinea-pig, and minimal in the rabbit.

2. Body epidermis is pigmented only in the guinea-pig.

3. Dopa-positive dendritic cells are present wherever melanin deposits are found.

4. Phagocytosis by dermal melanophores has been shown by intravitam injections of trypan blue.

5. Nuclear-capping occurs whenever melanin is found in epidermal cells or epidermal derivatives. It is not found in dermal melanophores.

6. The medullary banding of pigmented hair is a direct result of nuclear capping in the hair matrix cells.

I am indebted to Prof. A. Durward for advice and criticism; to Dr W. K. J. Walls for the photomicrographs; and to Mr R. K. Adkin for technical assistance.

REFERENCES

Becker, S. W. (1927). Melanin pigmentation; systematic study of the pigment of human skin and the upper mucous membranes with special consideration of pigmented dentritic cells. *Arch. Derm. Syph., N.Y.*, **16**, 259–290.

Becker, S. W., Praver, L. L. & Thatcher, H. (1935). An improved (paraffin section) method for the Dopa reaction. *Arch. Derm. Syph., N.Y.*, **31**, 190–195.

Billingham, R. E. (1948). Dendritic cells. *J. Anat., Lond.*, **82**, 93–109.

Billingham, R. E. (1949). Dendritic cells in pigmented human skin. *J. Anat., Lond.*, **83**, 109–115.

Billingham, R. E. & Medawar, P. B. (1947). Role of dendritic cells in the infective colour transformation of guinea pig's skin. *Nature, Lond.*, **160**, 61–62.

Bloch, B. (1929). The problem of pigment formation. *Amer. J. med. Sci.* **177**, 609–618.

Chase, H. B., Rauch, H. & Smith, V. W. (1951). Critical stages of hair development and pigmentation in the mouse. *Physiol. Zoöl.* **24**, 1–8.

Chase, H. B. & Smith, V. W. (1949). X-ray induced greying in the mouse with reference to melanoblasts. *Anat. Rec.* **105**, 533–534.

Hausman, L. A. (1928). The pigment granules of human head hair: a comparative racial study. *Amer. J. phys. Anthrop.* **12**, 272–283.

Ludford, R. J. (1924). I. Nuclear activity during melanosis: with special reference to melanin formation in a melanotic sarcoma. *J. R. micr. Soc.* **44**, 13–28.

Medawar, P. B. (1941). Sheets of pure epidermal epithelium from human skin. *Nature, Lond.*, **148**, 783.

Miescher, G. (1922). Cited by Meirowsky, E. (1940). In: A critical review of pigment research in the last hundred years. *Brit. J. Derm.* **52**, 210.

Rawles, Mary E. (1947). Origin of pigment cells from the neural crest in the mouse embryo. *Physiol. Zoöl.* **20**, 248–266.

Stearner, Sigrid Phyllis (1946). Pigmentation studies in salamanders, with especial reference to the changes at metamorphosis. *Physiol. Zoöl.* **19**, 375–404.

Steiner-Wourlisch, Aida (1925). Das melanotische Pigment der Haut bei der grauen Hausmaus (*Mus musculus* L.). *Z. Zellforsch.* **2**, 453–479.

EXPLANATION OF PLATE

Fig. 1. Whole-mount preparation of a sheet of pigmented epidermis from rat ear skin ('split skin'), seen from the dermal surface. The outlines of the epidermal cells can be faintly seen, with several Dopa-positive dendritic cells lying on their deep surface. (×480.)

Fig. 2. Frozen section of pigmented mouse ear skin, lightly stained with haematoxylin. Dense pigmentation of the upper dermis is present. (×130.)

Fig. 3. Vertical section of guinea-pig ear skin, stained with safranin. Pigmentation here is mostly epidermal, and especially marked along the basal epidermis. Nuclear capping is present. Dermal pigment is not so striking as in fig. 2. (×200.)

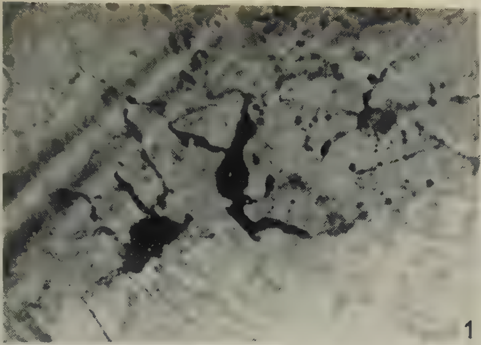
Fig. 4. Vertical section of pigmented rabbit ear skin, stained with haematoxylin and eosin. Pigment granules lie in the basal epidermis, and in the outer root sheaths of the hair follicles. No dermal pigment is present. (×360.)

Fig. 5. Vertical section of pigmented body skin of a guinea-pig. Parts of two dendritic cells are seen at the epidermo-dermal junction, and some melanin granules are seen within the basal epidermal cells. (×480.)

Fig. 6. Whole-mount preparation of a black mouse hair, unstained. Dark medullary bands, made up of melanin granules, are separated from each other by clear spaces. The cortex is narrow, but shows a few melanin granules. (×360.)

Fig. 7. Vertical section of rat skin, stained with haematoxylin and eosin. A hair follicle is cut longitudinally. It shows a pigmented hair matrix and medulla, with medullary banding. (×80.)

Fig. 8. Higher magnification of the deeper region of the medulla shown in fig. 7. Over the distal pole of the medullary nuclei, capping of pigment is seen. The few pigment granules in the cortex show a linear disposition, while the inner and outer root sheaths are unpigmented. (×480.)



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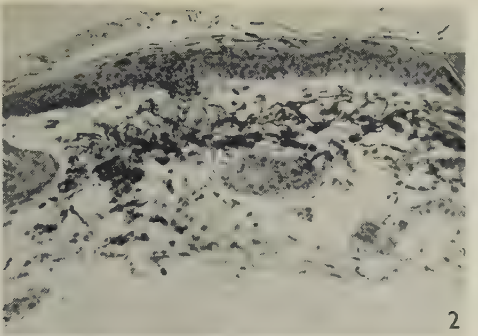

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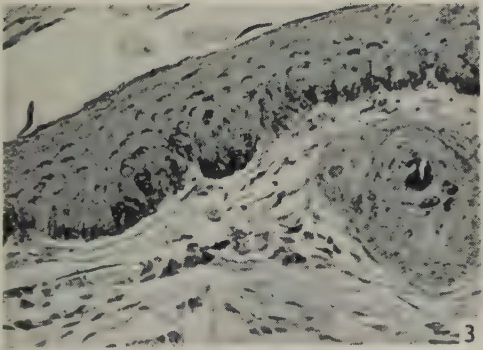

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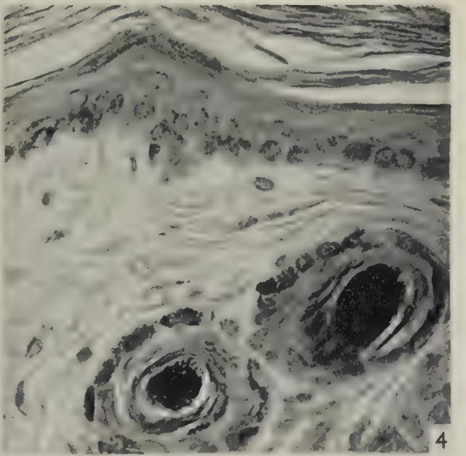

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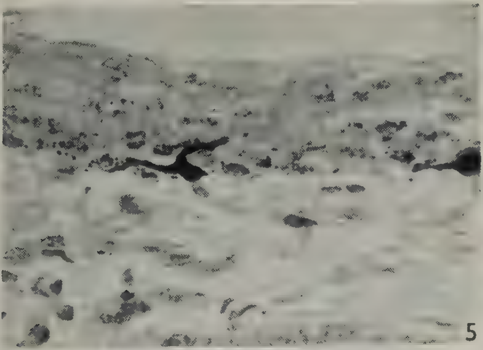

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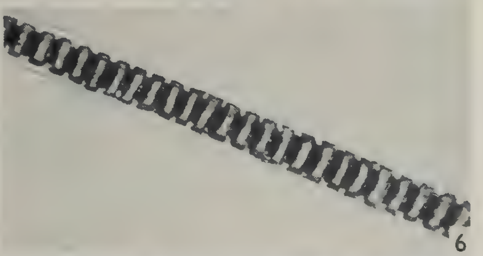

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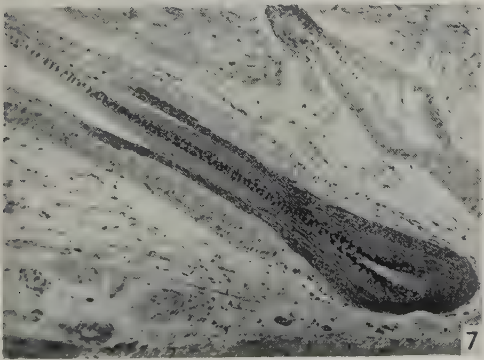

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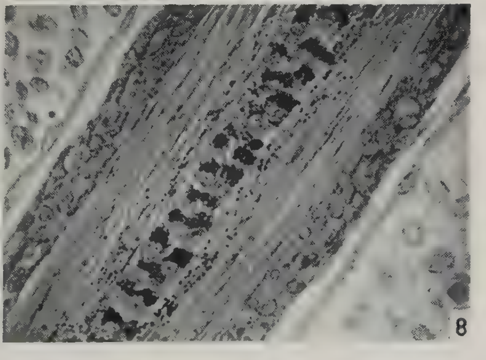

8

HOLMES—PATTERNS OF CUTANEOUS PIGMENTATION: RODENTS

THE AMPLITUDE OF PRONATION AND SUPINATION WITH THE ELBOW FLEXED TO A RIGHT ANGLE

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It has become increasingly evident that there is a definite need for fundamental information which will define the amplitudes of the normal movements of the joints of the human body. Apart from its intrinsic interest in the study of joint mechanics, such knowledge may also be of value to the clinician in assessing the functional capacity of his patient and to the engineer in designing machine controls. However, previously, this subject has received very little systematic investigation, and the present paper describes part of the work being undertaken in an attempt to fill this gap.

Previous work carried out by Darcus (1951) has indicated that measurements of the amplitude of pronation and supination of the hand taken on different occasions in the same subject vary considerably. A review of the relevant literature shows that the accounts of investigations carried out on the amplitude of this movement are, in general, confused and vague. The following study is part of an investigation aimed at determining accurately the amplitude of pronation and supination and the variations which occur in its measurement.

Generally accepted definitions of the terms pronation and supination are that, with the forearm horizontal, pronation is the movement which turns the palm of the hand to face downwards and supination that which turns the palm to face upwards. The movements are mainly dependent, when the elbow is flexed, on rotation of the radius about the longitudinal axis of the forearm at the radio-ulnar joints; associated with which there is a limited degree of flexion and extension at the elbow joint (Duchenne, 1867; Bryce, 1915; Brash & Jamieson, 1943; Johnston & Whillis, 1946) and a certain amount of rotation at the intercarpal joints (Cyriax, 1926).

The amplitude of pronation and supination has usually been measured either from the hand or from the distal end of the forearm with the arm adducted and the elbow flexed to 90°. Although there is this generally accepted convention concerning the posture in which the measurements are made, there is some disagreement on the point from which pronation and supination should be measured. Pronation and supination are either measured separately from an arbitrary neutral position or the total amplitude is recorded from full supination to full pronation and vice versa; both systems have not been used on the same group of subjects. The neutral position, when specified, is usually described as the position in which the plane of the hand is vertical and the palm faces medially. This neutral position is either given the angular value of 0° (West, 1945; Darcus, 1951) or 90° (Clark, 1920; Robinson, 1921; Wiechec & Krusen, 1939).

Work on the amplitude of joint movement in man appears to have received its main impetus from the large number of orthopaedic patients resulting from the 1914–18 war. About this time, methods and instruments for the measurement of the amplitude of joint movement were first developed.

Cleveland (1918) measured pronation and supination by estimating the amplitude visually, and recording the limits of movement on a circular chart. The arc of movement was magnified by a rod gripped in the hand of the subject. Rosen (1922) assumed that for clinical purposes any deviation from the normal amplitude could be estimated sufficiently accurately by using the thumb and hand as an indicator without the use of any apparatus. Others, however, have stressed the need for a more accurate method of measurement which would not be too elaborate for clinical work.

The first instrument to measure pronation and supination was designed by Amar (Fox, 1917). It consisted essentially of a horizontal support for the forearm and a centrally pivoted handle. Projecting from the handle was a pointer which indicated the amplitude on a fixed vertical semicircular scale. Darcus (1951) used a similar method but had no support for the forearm. The apparatus devised by Gifford (1914) differed in that the movement was measured from an adjustable clamp around the wrist. Clark (1921) described an apparatus which consisted solely of a protractor scale arching over the distal end of the forearm. The amplitude of movement was determined by projecting by eye a line passing through the radial and ulnar styloid processes on to the scale. The method of Silver (1923) was similar in principle, except that the protractor scale was positioned at right angles to the longitudinal axis of the little finger and the plane of the hand was used as an indicator. Dorinson & Wagner (1948) used a caliper method for measuring pronation and supination. One shaft of the caliper was allowed to rest on a table at right angles to the forearm, whereas the other shaft was vertical. The ulnar border of the wrist corresponded to the union of the two shafts. The upright shaft was moved by the flexor aspect of the wrist in pronation and by the extensor aspect during supination. Glanville & Kreezer (1937) used a pendulum goniometer for studying pronation and supination. The goniometer was grasped by means of a strap, and as the hand was rotated the scale of the instrument moved with it, while a weighted pointer pivoted at the centre of the scale remained vertical. Patrick (1946) recommended a similar instrument for clinical work; the main difference being that the strap was replaced by a wooden handle.

A summary of the figures given for the amplitude of pronation and supination is shown in Table 1. The text-book estimates are presented without giving any reference as to their source, and the majority of values given in original papers, which are alleged to represent an approximate normal amplitude, do not indicate the limits of normal variations nor the size or the characteristics of the groups on which the average values were determined.

METHODS

The hand-grip arthrometer which was used for the measurement of the amplitude of pronation and supination at the hand was adapted from an instrument which had been primarily designed to measure the forces exerted in pronation and supination (Darcus, 1951). It consists essentially of a handle which the subject grips. This is fixed to one end of a spindle which is free to rotate around its longitudinal axis. The line of this axis when projected to the hand passes through the middle finger. This corresponds to that reported by Duchenne (1867) as being the usual axis for the movements of pronation and supination, although it may vary with the task being performed. A pointer attached to the hand-grip spindle indicates the angular displacements on a large circular scale on the back of the metal plate supporting the spindle. In this position, the pointer cannot be seen by the subject, so that he has no objective means of accurately assessing his performance. This was done to reduce the possible effects of motivation that knowledge of the results might produce. Accuracy of recording the extreme position of the pointer was improved by adding a marker which is moved by the pointer.

Table 1. *Average amplitude of pronation and supination as reported by different authors*

Source	No. of subjects	No. of observations on each	Side	Average amplitude in degrees		
				Pronation	Supination	Total
Macalister (1889)	—	—	—	—	—	165
Muller (1929)	—	—	—	—	—	140–160
Mainland (1947)	—	—	—	—	—	*c.* 135
Johnston & Whillis (1949)	—	—	—	—	—	160–170
Appleton *et al.* (1948)	—	—	—	—	—	>135
Albee & Gilliland (1920)	—	—	—	—	—	225
Clark (1920)	—	—	—	50	90	140
Rosen (1922)	—	—	—	90	90	180
Silver (1923)	—	—	—	<80	<80	<160
Frescoln (1929)	—	—	—	>90	>90	>180
Coulter & Molander (1935)	—	—	—	—	—	<180
Dorinson & Wagner (1948)	—	—	—	80	70	150
				(measured at wrist)		
Sinelnikoff & Grigorowitsch (1931)	116	3	—	80–132	30–102	175
Glanville & Kreezer (1937)	10	3	R.	91	99	190
			L.	93	101	194
Darcus (1951)	3	10	R.	68	110	178
					(arm adducted)	
			R.	74	115	189
					(arm flexed)	
			R.	66	124	190
					(arm abducted)	

The wrist-cuff arthrometer was designed for the measurement of radio-ulnar movement with the elbow flexed (Figs. 1 and 2). This instrument consists of a tubular metal rod, fixed at one end to the top of the metal plate of the hand-grip arthrometer so that it is parallel to the spindle. Attached to this metal rod, parallel to and at an adjustable distance from the plate, is a metal frame in which rotates a circular scale. The lower end of the radius is fixed within this scale by two shaped pieces of metal lined with rubber which can be tightly and comfortably clamped in position. The wrist-cuff and scale can be adjusted so that the subject can also grasp the hand grip, so that recordings of movements of the hand and of the distal end of the radius can be made simultaneously (Fig. 3).

For purposes of comparison, Patrick's modification of the pendulum type goniometer was also used.

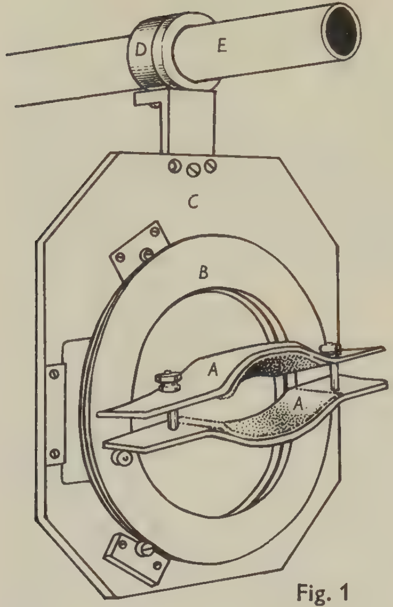

Fig. 1

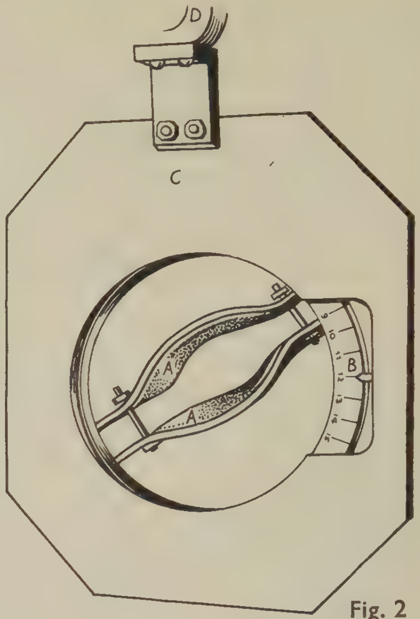

Fig. 2

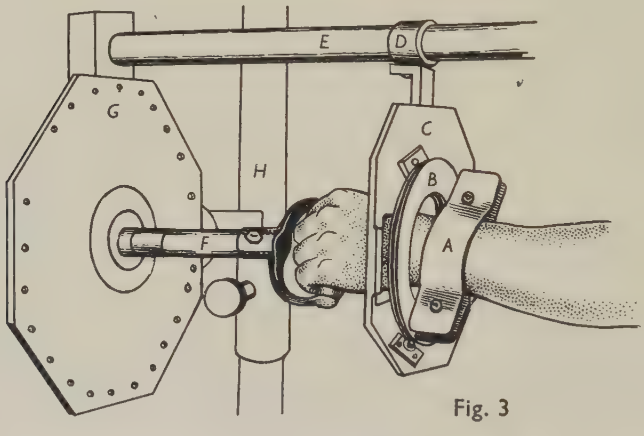

Fig. 3

Figs. 1 and 2. Front and back views of wrist-cuff arthrometer. Fig. 3. Wrist-cuff and hand-grip arthrometers in position for simultaneous recordings. The wrist-cuff arthrometer consists of two rubber-lined metal plates (*A*), which can be clamped around the wrist. One of these plates is fixed at either end to a circular scale (*B*), which rotates in a metal frame (*C*). This frame is attached by a metal ring (*D*), which can be moved along a tubular metal rod (*E*). This rod is mounted on the metal plate of the hand-grip arthrometer (*G*). The hand-grip arthrometer consists of a handle connected to a spindle (*F*), which can rotate in bearings in the centre of a metal plate (*G*). A pointer projecting from the spindle moves over a large circular scale on the back of the plate. The whole apparatus is attached to an adjustable support (*H*).

At the beginning of each experiment, the apparatus and the purpose of the experiment were explained to the subjects. Throughout the experiments, the subjects had no knowledge of their results and, while efforts were made to maintain their interest, no positive incentive was provided. The subjects were instructed, and frequently reminded, to make movements of maximal amplitude.

All experiments were conducted with the subject seated. The chair was adjusted so that the subject sat in a comfortable erect position with the thighs horizontal and the legs vertical, and the apparatus was adjusted so that the arm was adducted to the side of the body and the elbow flexed to 90°. The height of both the chair and the apparatus was recorded to assist in reproducing the relative position of the subject and apparatus on successive occasions.

One observer recorded the readings and a second observer watched the subject for gross changes in posture and for 'trick' movements. The age, sex, occupation, preferred hand and history of any injury to arm or forearm were noted for each subject. In all experiments, the total amplitude of pronation and supination was measured. The angle subtended by the two extremes of excursion of the joint was recorded. Ten readings were taken at 10 sec. intervals on one or more days and, in the majority of cases, on the right and left sides. It was originally intended that no neutral point should be used. However, in order that a comparison could be made with the results of Glanville & Kreezer (1937) and for the possible value it may have in practical problems, the majority of the results have also been analysed into separate movements from a neutral position. In measuring the amplitude of pronation and supination with the hand-grip arthrometer and Patrick's goniometer, the neutral position was taken to be that in which the plane of the hand is vertical and the palm is facing medially. Using the wrist-cuff arthrometer, the neutral position was that in which the distal ends of the radius and ulna are in the same vertical plane. Movement in the direction of pronation was designated as minus and in the direction of supination as plus.

The measurements obtained were subjected to statistical analysis. When using the 't' test, P had to be less than 0·01 before any statistical significance was attached to the differences found, or, in other words, the probability that a difference might be due to chance had to be less than one in 100.

The study has included the measurement of the amplitude of pronation and supination using a hand-grip arthrometer, a comparison between the measurements of the amplitude of pronation and supination using the hand-grip and wrist-cuff arthrometers, a comparison between the measurements of the amplitude of pronation and supination using the hand-grip and wrist-cuff arthrometers and Patrick's goniometer and the determination of the effect of the recording apparatus on the amplitudes.

THE MEASUREMENT OF THE AMPLITUDE OF PRONATION AND SUPINATION AT THE HAND USING THE HAND-GRIP ARTHROMETER

In this experiment, thirteen male subjects between the ages of 15 and 30 years were used. All were right-handed. Five had previously sustained a fracture of the radius or ulna or both. They were chosen at random from the academic and technical staffs of the Department of Anatomy and from naval ratings attached to the Unit. Measurements were made on both the right and left sides and repeated on five consecutive days; the right side being tested first on alternate days.

Observations

The amplitude of movement (Table 2). The average total amplitude for the right and left sides was 167·2° with values varying between 145·3 and 188·0. The average position of the mid-point of the movement was 20·8° on the 'supination' side of the neutral point. The mean amplitude of pronation from the neutral point was −62·8° and that of supination +104·4°.

The effect of age. In order to determine whether or not, in the age range tested, there was any significant association of the amplitude with age, the results of the four 'uninjured' subjects between the ages of 15 and 19 years were compared with the four between the ages of 21 and 30 years. The average amplitude of the younger group was 173·4°; 5·9° more than for the older group, but this difference was not statistically significant.

Table 2. *Mean amplitude of pronation and supination. Average of fifty observations on each side in each subject. The figures in italics indicate the amplitude of the injured side*

	Right		Left	
Age	Amplitude (°)	S.D.	Amplitude (°)	S.D.
15	152·2	6·82	151·5	8·85
16	187·1	17·93	188·0	19·01
17	178·7	18·17	180·1	13·82
19	170·7	13·40	179·2	13·33
21	170·5	28·02	184·5	23·15
23	185·8	26·19	177·3	15·49
27	158·4	11·89	162·0	12·09
30	153·1	15·73	148·5	18·89
20	*145·3*	5·18	161·2	6·47
23	162·1	7·55	*167·1*	9·35
29	*162·0*	6·75	180·6	7·74
29	*163·6*	7·05	158·1	5·91
30	164·5	9·24	*156·2*	10·75
Mean	165·7	12·55	168·8	13·40

The amplitude on the right and left sides (Table 2). The average amplitude of the measurements from all subjects was slightly larger on the left than on the right (1·9 %). Individually, the readings on the left exceeded those on the right in eight subjects, whereas in the other five the reverse was found. In seven of the subjects, these differences, the largest of which was 11·5 %, were statistically significant. It should be noted, however, that five of these belonged to the 'injured' group. In only four of the seven were the differences in the same direction on each of the 5 days; in the other three the difference was the reverse of that of the means for the 5 days on one occasion. As all the subjects were right-handed, there is apparently no relation between the amplitude of movement and the preferred hand. It was shown statistically, using a rank correlation method, that those subjects having a small amplitude on the right side tended also to have a small amplitude on the left side.

The effect of previous injury. The average amplitude of movement for the right and left sides of the five subjects who had previously sustained a fracture of either the radius or ulna or both was less than that of the uninjured group, but the difference (9·3°) was not statistically significant. Three of the 'injured' subjects consistently showed a significantly lower amplitude on the injured side (average 9·1 %). The other two, however, showed a significantly lower amplitude on the uninjured side (average 3·2 %), but these differences were not consistent from day to day.

The variation between successive readings and successive groups of readings. There was considerable variation in the same individual between the means of the ten readings taken on each of the 5 days and between the ten readings taken on the same day. The variations within a group or between groups shows no consistent trend; that is, there is no regular decrease or increase in the readings during the experimental period. Neither is there any correspondence between the variation found on the two sides on any one day, although the over-all variation on the two sides is similar.

Use of the apparatus. During the course of the experiment, it was observed that slight changes in the grip of the handle of the arthrometer could lead to alterations in the amplitude of movement. This was particularly marked at the pronation end of the movement, where the subject was tempted to loosen his grip and twist the handle with his fingers.

THE MEASUREMENT OF THE AMPLITUDE OF PRONATION AND SUPINATION WITH THE ELBOW FLEXED USING THE HAND-GRIP AND WRIST-CUFF ARTHROMETERS

From the experience gained in the previous experiment, it was considered that variations in the amplitude of pronation and supination in the same individual on different occasions were possibly due to alterations in the grip. To investigate this point, the wrist-cuff arthrometer was developed and a comparison was made between measurements taken at the hand and at the lower end of the radius.

Four female subjects between the ages of 19 and 27 years were used. The procedure was the same as in the first experiment.

Observations

The amplitude of movement (Table 3). The mean total amplitude measured from the hand-grip arthrometer in each subject was 182·7°; 26·5° greater than that measured simultaneously from the wrist-cuff arthrometer. The amplitudes of pronation and of supination measured by the two methods cannot be compared directly, because the neutral points used in each case do not correspond. Although no data were available when these experiments were carried out, it has since been shown on another group of subjects that, when the wrist-cuff arthrometer registers zero, i.e. when the distal ends of the radius and ulna are in the same vertical plane, the hand-grip arthrometer indicates an average position of +11° (i.e. 11° to the 'supination' side of the zero as defined for the hand-grip arthrometer). This figure represents the average of readings from six subjects, with individual values ranging from +5° to +18°. If it can be assumed that such a difference existed in the subjects of the experiment, the amplitudes of pronation and of supination may be rendered comparable by adding 11° to the pronation and subtracting 11° from the supination values obtained from the hand-grip arthrometer readings. The difference between the adjusted values for supination is less than 1°, whereas the difference between those for pronation is 27°. Thus, the increase in the amplitude using the hand-grip arthrometer appears to occur mainly at the pronation end of the range. It was here that in the first experiment it was noticed that there was a loosening of the grip, and it is believed that this accounts for at least some of the difference. However, it is possible that part may be due to a certain degree of rotation at the joints of the wrist and the hand. This is investigated in the next experiment.

Table 3. *Amplitude of pronation and supination as measured from the hand-grip arthrometer and from the wrist-cuff arthrometer. Average of fifty observations on each side*

		Amplitude (°)			Variation within days		Variation between days		Total variation	
Age of subject	Side	Hand-grip (*H*)	Wrist-cuff (*W*)	Difference (*H*−*W*)	*H*	*W*	*H*	*W*	*H*	*W*
19	R.*	177·1	148·3	28·8	9·47	5·20	4·45	2·66	10·45	5·84
	L.	178·0	145·0	33·0	6·99	3·17	5·61	12·77	8·96	13·15
21	R.*	156·7	148·9	7·8	5·84	3·10	11·52	6·96	12·92	7·31
	L.	164·7	159·6	5·1	6·19	2·88	7·91	8·05	10·05	8·55
22	R.*	201·8	159·0	42·8	8·44	3·52	8·85	6·15	12·24	7·08
	L.	192·0	156·3	35·7	9·51	5·04	7·73	6·35	12·26	8·11
27	R.	190·2	161·4	28·8	7·86	3·27	7·88	8·71	11·13	9·30
	L.*	201·4	171·2	30·2	9·95	4·66	14·21	12·02	17·32	12·89
Mean	R.	181·5	154·4	27·0	—	—	—	—	—	—
	L.	184·0	158·0	26·0	—	—	—	—	—	—

* Preferred hand.

When the total amplitudes, as measured from the hand-grip arthrometer, are compared with those in the previous experiment in an equivalent age group, no significant difference is found.

The amplitude on the right and left sides (Table 3). Using both methods of measurement, the average amplitude for the left side was slightly greater (2·5 %) than on the right. Using the wrist-cuff arthrometer, the readings were greater on the right than on the left in two subjects, whereas in the other two the reverse was found. Using the hand-grip arthrometer, the readings were greater on the left side in three cases out of four. With both the hand-grip and wrist-cuff arthrometers, the differences proved to be statistically significant.

Variation between successive readings and successive groups of readings (Table 3). The degree of variation within each group of readings is significantly lower with the wrist-cuff arthrometer than with the hand-grip arthrometer. The variation between successive groups is less with the wrist-cuff arthrometer in five cases out of eight, and the total variation is less in seven cases out of eight. In general, therefore, the wrist-cuff arthrometer gives more reproducible values.

A COMPARISON BETWEEN THE MEASUREMENTS TAKEN SIMULTANEOUSLY WITH THE HAND-GRIP AND WRIST-CUFF ARTHROMETERS WITH MEASUREMENTS TAKEN ON PATRICK'S GONIOMETER

The same procedure was adopted as in the previous experiments except that the measurements were taken on only one day.

Observations (Table 4)

For the two subjects used, the readings were smallest with the wrist-cuff arthrometer and largest with the hand-grip arthrometer, with those from Patrick's goniometer occupying an intermediate position.

Table 4. *Amplitude of pronation and supination as measured from (A) the hand-grip arthrometer, (B) Patrick's goniometer and (C) the wrist-cuff arthrometer. Average of ten observations on each side in two subjects*

		Right		Left	
	Subject	Amplitude (°)	S.D.	Amplitude (°)	S.D.
A	I	177·0	6·79	187·7	4·06
B	I	166·4	2·76	173·7	3·97
C	I	149·1	3·84	162·1	2·56
A	II	170·6	5·85	163·0	3·46
B	II	167·1	4·20	162·4	2·59
C	II	156·7	4·47	148·5	3·06

The average difference of 20·5° between the hand-grip and wrist-cuff arthrometer readings was of the same order as found in the previous experiment. The average difference between the readings using the wrist-cuff arthrometer and Patrick's goniometer was 13·3°, and between Patrick's goniometer and the hand-grip arthrometer was 7·2°. Although the experiment was limited, the results suggest that, when the amplitude of pronation and supination is measured from the hand, the movements at the radio-ulnar joints are supplemented by rotation occurring at the joints of the wrist and hand. As Patrick's goniometer has to be supported by the hand as well as rotated, no alterations of grip are possible when effecting a maximal movement, so that hand movements cannot supplement the rotation.

The variations in the groups of ten readings were of the same order when using the wrist-cuff arthrometer and Patrick's goniometer, whereas when using the hand-grip arthrometer they were larger. This gives further support to the suggestion that part of the variation of readings found in the first experiment was due to the way in which the apparatus was used.

THE DETERMINATION OF THE EFFECT OF THE WRIST-CUFF ARTHROMETER ON THE AMPLITUDE OF PRONATION AND SUPINATION

The experiment was undertaken to determine whether or not the wrist-cuff arthrometer interfered in any way with the movements measured. For this purpose, the amplitude of pronation and supination was measured as follows on five subjects on the right side only:

(1) Using the hand-grip arthrometer alone.

(2) Using Patrick's goniometer alone.

(3) Using the wrist-cuff arthrometer alone.
(4) Using the wrist-cuff and hand-grip arthrometers simultaneously.
(5) Using the wrist-cuff arthrometer and Patrick's goniometer simultaneously.

Ten consecutive readings were taken in each case on one occasion only.

Observations (Table 5)

The statistical analyses of the results indicate that the wrist-cuff arthrometer does not restrict the movement of pronation and supination when the elbow is flexed; neither does the use of the hand-grip arthrometer nor that of Patrick's goniometer significantly affect readings taken on the wrist-cuff arthrometer.

Table 5. *Amplitude of pronation and supination as measured using different experimental procedures. Average of ten observations on the right side*

	Hand-grip		Patrick's		Wrist-cuff		
Subject	Alone	With wrist-cuff	Alone	With wrist-cuff	Alone	With wrist-cuff	With Patrick's
1	165	164	166	160	156	149	141
2	166	167	173	164	140	135	136
3	183	172	168	165	152	155	149
4	240	225	170	161	140	150	137
5	190	198	171	179	163	158	158
Mean	188·8	185·2	169·6	165·8	150·2	149·4	144·2

Table 6. *Summary of average amplitudes of pronation and supination as measured from (A) the hand-grip arthrometer, (B) Patrick's goniometer and (C) the wrist-cuff arthrometer*

			Amplitude (°)	
Experiment	Number of subjects	Total number of readings	Right	Left
A 1	13	650	165·7	168·8
2	4	200	181·5	184·0
3	2	20	173·8	175·3
4	5	100	187·0	—
			Mean 173·8	
B 3	2	20	166·7	168·1
4	5	100	167·7	—
			Mean 166·9	
C 2	4	200	154·4	158·0
3	2	20	152·9	155·3
4	5	150	147·9	—
			Mean 156·3	

The results support the finding in the previous experiment in that the readings were smallest with the wrist-cuff arthrometer and largest with the hand-grip arthrometer, with those from Patrick's goniometer occupying an intermediate position.

DISCUSSION

Comparison with previous results (Table 7)

The only results with which those of the present series of experiments can be compared are those of Glanville & Kreezer (1937). These workers, however, do not give figures for the total amplitude, and it is possible to obtain only the average total amplitude from the average amplitude of pronation and of supination. The range of values cannot be derived, as it cannot be assumed that a subject with a maximum or minimum amplitude in one direction necessarily has either a maximum or minimum amplitude in the reverse direction.

Table 7. (*A*) *Mean amplitude of pronation and of supination as measured from the hand-grip arthrometer. Neutral point with the handle vertical* (0°). (*B*) *The figures of Glanville & Kreeser* (1937) *obtained with a pendulum goniometer are included for comparison*

			Right			Left		
	No. of subjects	Total no. of observations	Amplitude (°)	Range	S.D.	Amplitude (°)	Range	S.D.
					Pronation			
A	13	650	63	49–84	10·5	62	49–75	8·6
B	10	30	91	59–139	25·8	93	69–135	20·7
					Supination			
A	13	650	102	86–122	11·1	106	90–121	10·0
B	10	30	99	81–114	11·0	101	74–114	10·8

The fact that their average total amplitude is considerably higher than those reported in this paper may possibly be explained by the different methods used. However, our results indicate that for the measurement of pronation and supination the hand-grip arthrometer gives higher readings than Patrick's goniometer; the latter being similar in principle to the pendulum arthrometer used by Glanville & Kreezer. If this was one of the main variables, therefore, one would have expected their values to be smaller rather than larger than those found in the present investigation. One can only presume that the differences are due, at least in part, to the capabilities of the subjects used.

Despite the fact that we did not measure the amplitude of the movement in each direction from a neutral point, it was possible to obtain this information for each individual. When these figures for pronation and supination using the hand-grip arthrometer are compared with those of Glanville & Kreezer from a similar neutral point, the most striking fact is that, whereas the figures for supination are very similar, our figures for pronation are much smaller and show much less individual scatter. There appears to be no satisfactory explanation of this, except, perhaps, that movements measured separately from a neutral point are liable to be inaccurate (see below).

Measurements of the amplitude of movements from a neutral point

There appears to be very little advantage gained by measuring the amplitudes of pronation and supination from a neutral point. The only object of this procedure appears to be the possible practical application in the design of machine controls. For instance, it may be necessary to know the distance a control can be rotated by pronation and supination of the hand from a fixed point. This information cannot be obtained from figures merely representing the total amplitude, as these contain no indication of the orientation of the movement in space. However, perhaps more valuable data, applicable to this type of problem, may be provided by knowing the individual deviation of mid-points of the total amplitude from a defined reference point. This can be readily calculated from the results of the total amplitude, providing that the angular measuring scale can be related to a known plane. For example, our apparatus was calibrated so that 0° on the scale was in the vertical plane passing through the axis of the movement.

To measure the amplitude of rotation in either direction from a neutral point is creating an artificial division of movement. For instance, the complete movement of supination is from full pronation to full supination and not from some arbitrary intermediate point to full supination. To take measurements separately from a neutral point breaks up the movement unnecessarily. It is also probable that there will be differences between the total amplitude recorded as a complete movement and that obtained by adding the amplitudes of pronation and supination measured independently from a neutral point. Furthermore, it is very difficult to specify a neutral point accurately and even more difficult to reproduce this for different subjects. Also, with different methods, although the description of the neutral point may be apparently the same, its relation to the total movement may be different. For example, the neutral point for pronation and supination using the hand-grip arthrometer, which is taken with the handle vertical, is on the average 11° nearer the full pronation end of the movement than that using the wrist-cuff arthrometer, which is taken with the wrist-cuff vertical. Thus, if the hand-grip method is used, the proportion of pronation (from a neutral point with handle vertical) to the total movement is less than when using the wrist-cuff arthrometer. This difference between the plane of the handle gripped by the hand and the plane passing through the distal ends of the radius and ulna is explained by the relative movements of the carpo-metacarpal joints of the fingers. If the handle is loosely gripped, the plane of the handle and that passing through the distal ends of the radius and ulna coincide. When the handle is gripped firmly without movement of the forearm, the handle moves in the direction of supination due to flexion of the fourth and fifth carpometacarpal joints (Grant, 1948).

Another possible disadvantage of using a neutral point is that, owing to slight shifts of the apparatus in relation to the part being studied, this point varies from measurement to measurement on the same subject and from subject to subject. This may partly explain the greater range of variation in the individual measurements of pronation and supination. A further source of variation is that, although the total amplitude may be similar in two subjects, the distribution of the movements on either side of a central point may vary considerably. This is indicated by the finding that there is a wide range of mid-points of the individual total amplitudes in relation to a neutral point. Similar variations have been found by Darcus (1951).

For clinical use, there seems to be no adequate reason for dividing a movement. No purpose is served, as skeletal or neuro-muscular disorders do not, as a rule, affect differently the amplitude in one or other direction from a neutral point, but affect the total movement. The total amplitude will give a much better index of the impairment of joint function and of the course of the clinical condition.

In summary, it is clear that measuring the amplitude from a neutral point leads to an inadequate presentation of data and increased experimental difficulties, and its practical application is limited.

Variation between successive readings and successive groups of readings

The only other workers to comment on the marked variation between successive readings of maximum joint movement in any one individual are Cobe (1928) and Hewitt (1928). In studying wrist movements, Hewitt found that even in the subject who co-operated willingly there was an amazing fluctuation in the amplitudes; the value on one occasion sometimes being more than double that on another for a particular movement. These variations, she considered, were not due to errors on her part or to defects in her apparatus. On the other hand, none of the variations in our experiment reached this magnitude.

The cause of these variations is obscure and probably depends on a large number of factors, some of which are uncontrollable. Some of the variations can no doubt be attributed to the experimental procedure. Although every effort was made to position the subject in exactly the same position in relation to the apparatus on successive occasions, it is conceivable that slight deviations of posture did occur, which might have led to an increase or decrease in the amplitude recorded. This, however, should not be so important in successive readings on one subject. Variations in the first experiment using the hand-grip dynamometer might be explained by changes in grip during the recordings. This is supported by the fact that variations within a group of readings were decreased by approximately 50 % by the use of the wrist-cuff arthrometer. It is also possible that the way in which the apparatus was moved by the subject varied from time to time, although obvious alterations due to 'trick' movements serving to increase the amplitude, such as abduction of the arm during pronation, were prevented by the observer. Cobe (1928) and Hewitt (1928) concluded that the variations they found were due to the fact that the movements they were testing were seldom normally carried out to a maximum. Thus, when a maximum effort is required, the movements are not under sufficient control and may, therefore, be performed with uncertainty. It is interesting to note that no less variation was found on the right side, in right-handed subjects, than on the left. Whether or not training has any effect on the amplitude of movement has not been tested specifically, but the absence of general increase in ten readings of each group and the mean readings of successive groups suggests that it is not an important cause of variation, at least in relatively short-term experiments. Hewitt (1928) concluded, however, that prolonged exercise over 4 years increases the amplitude of movement of the wrist joint. The absence of a decrease in the variation within groups of ten readings on successive days, indicates that the amount of training afforded in the present experiments does not affect the accuracy with which successive movements are repeated.

During unresisted movements, there is activity in the antagonist muscles representing a certain level of tension exerted by these muscles. It is possible that alterations in this tension may affect the arc through which the joint is moved; the less the activity in the antagonists, the more movement and vice versa. It might also be suggested that the sensitivity of the stretch receptors varies, so that the antagonistic muscles reflexly contract to limit the movement at different phases on different occasions. The reason for these possible changes in the muscular activity of the antagonists is not clear. It may be local effect, such as change in temperature, or a central effect. Further light might be thrown on this aspect by electromyographic records of the muscles while effecting maximum voluntary movements.

Probably, however, the most potent factors causing variation are psychological in nature. It is extremely difficult, if not impossible, to keep the level of motivation of a subject constant, and thus his will to do his 'best' changes from time to time. The incentive which might be provided by knowing the results of his efforts was withheld from the subject. This is probably responsible for some of the variations, as the subject had no objective means of determining his performance and had to rely on subjective impressions, which may not have been dependable. Also, the attention of the subject to the task in hand fluctuated; some subjects being readily distracted by such occurrences as people walking through the laboratory or past the window or the telephone ringing in an adjacent room. Although distractions were minimized as far as possible, when they did occur, they appeared to have an adverse effect on the performance of the individual. It is interesting to note in this respect that the most willing and co-operative of the subjects in general showed smaller variations.

One can only conclude from the variations found in this and in other experiments that, even if everything is done to reduce to a minimum variations due to extraneous influences, a certain degree of variability must be accepted in all measurements of maximum values in man. The presence of these variations also serves to emphasize the hazards of drawing conclusions from a single measurement of the amplitude of any movement, especially in clinical work, where such measurements may be accompanied by discomfort or some other limiting factor.

Limits of individual variations

The limits of individual variation are large, and it is therefore difficult to give a 'normal value'. Using the hand-grip arthrometer, even on the limited group of subjects used, the 95 % distribution range was 138–211°. This may be partly accounted for by different subjects using supplementary movements of the hand and fingers to a widely varying degree. Using a more accurate method, i.e. the wrist-cuff arthrometer, which eliminates these variables, the 95 % distribution was 133–169°. Compared with the individual variation, the variation between the amplitude from the right and left sides is very small. When a standard for comparison is needed, therefore, the contralateral limb, if normal, supplies a better standard than any 'normal' value.

SUMMARY

1. The available literature on the measurement of the amplitude of pronation and supination is reviewed.

2. A wrist-cuff arthrometer, which has been designed to measure the amplitude of radio-ulnar movement at the distal end of the radius, is described.

3. Measurements of the amplitude of pronation and supination with the elbow flexed to 90° have been recorded with a hand-grip arthrometer (twenty-four subjects), the wrist-cuff arthrometer (eleven subjects) and Patrick's goniometer (seven subjects). In the majority of cases, both right and left sides have been studied.

4. The mean amplitudes obtained have been summarized in Table 6. The largest readings were obtained with the hand-grip (protractor type) arthrometer which, besides recording rotation of the radio-ulnar joints, also measures supplementary movements of the hand and wrist. Intermediate values were recorded with a pendulum-type arthrometer, which excluded hand movements but allowed rotation at the intercarpal joints. The lowest readings were recorded using the wrist-cuff arthrometer, which measures almost exclusively movements about the radio-ulnar joints.

5. No significant association between either age, sex or previous injury and amplitude was found in the subjects studied.

6. Although differences were found between the amplitudes measured on the right and left sides of the same individual, these usually did not reach a significant level.

7. Wide variation occurred between readings taken successively on the same day and from day to day. Possible reasons for these variations are discussed.

8. The results of the present experiments are compared with those of other workers, and the methods of measuring and recording the amplitudes of pronation and supination are discussed.

9. It is concluded that, owing to the wide individual variation, 'normal' figures for the amplitude of pronation and supination are only of limited value. In clinical work, the findings of these experiments indicate that, in cases of injury to one limb, readings from the opposite side are a more reliable yardstick.

REFERENCES

ALBEE, F. H. & GILLILAND, A. R. (1920). Metrotherapy, or the measure of voluntary movement. *J. Amer. med. Ass.* 75, 983–986.

APPLETON, A. B., HAMILTON, W. J. & SIMON, G. (1948). *Surface and Radiological Anatomy*, 2nd ed. pp. 46–70. Cambridge: Heffer and Sons.

BRASH, J. C. & JAMIESON, E. B. (1943). *Cunningham's Textbook of Anatomy*, 8th ed. pp. 329–353. London: Humphrey Milford.

BRYCE, T. H. (1915). *Quain's Elements of Anatomy*, 11th ed., vol. IV, part I, pp. 220–245. London: Longmans Green and Co.

CLARK, W. A. (1920). A system of joint measurements. *J. orthop. Surg.* 2, 687–700.

CLARK, W. A. (1921). A protractor for measuring rotation of joints. *J. orthop. Surg.* 3, 154–155.

CLEVELAND, D. E. H. (1918). Diagrams for showing the limitation of movements through joints. *J. Canad. med. Ass.* 8, 1070–1076.

COBE, H. M. (1928). The range of active motion at the wrist of white adults. *J. Bone Jt. Surg.* 10, 763–774.

COULTER, J. S. & MOLANDER, C. O. (1935). Therapeutic exercise. *J. Amer. med. Ass.* 104, 118–120, 213–219.

CYRIAX, E. F. (1926). On the rotary movements of the wrist. *J. Anat., Lond.*, 60, 199–201.

DARCUS, H. D. (1951). The maximum torques developed in pronation and supination of the right hand. *J. Anat., Lond.*, 85, 55–67.

DORINSON, S. M. & WAGNER, M. L. (1948). An exact technic for clinically measuring and recording joint motion. *Arch. phys. Med.* 29, 468–475.

Duchenne, G. B. A. (1867). *Physiology of Motion*, p. 101. (Translated by Kaplan, E. B., 1949.) Philadelphia: Lippincott.

Fox, R. F. (1917). Demonstration of the mensuration apparatus in use at the Red Cross clinic for the physical treatment of officers. *Proc. R. Soc. Med.* **10**, Sect. Baln. Climat. 63–68.

Frescoln, L. D. (1929). Range of bodily movements. *Med. Times, N.Y.*, **57**, 197–198.

Gifford, H. C. (1914). Instruments for measuring joint movements and deformities in fracture treatment. *Amer. J. Surg.* **28** (1st series), 237–238.

Glanville, A. D. & Kreezer, G. (1937). The maximum amplitude and velocity of joint movements in normal male human adults. *Hum. Biol.* **9**, 197–211.

Grant, J. C. B. (1948). *A Method of Anatomy*, 4th ed. p. 198. London: Baillière, Tindall and Cox.

Hewitt, D. (1928). Range of active motion at the wrist of women. *J. Bone Jt. Surg.* **10**, 775–787.

Johnston, T. B. & Whillis, J. (1946). *Gray's Anatomy*, 29th ed. p. 445 and p. 484. London: Longmans Green and Co.

Macalister, A. (1889). *A Textbook of Human Anatomy*, p. 42 and pp. 146–154. London: Griffin.

Mainland, D. (1947). *Anatomy*, p. 226. London: Hamish Hamilton.

Muller, G. P. (1929). *Applied Anatomy*, 8th ed. p. 306. London: Lippincott.

Patrick, J. (1946). A study of supination and pronation, with especial reference to the treatment of forearm fractures. *J. Bone Jt. Surg.* **28**, 737–748.

Robinson, W. H. (1921). Joint range. *J. orthop. Surg.* **3**, 41–51.

Rosen, N. G. (1922). A simplified method of measuring amplitude of motion in joints. *J. Bone Jt. Surg.* **4**, 570–579.

Silver, D. (1923). Measurement of the range of motion in joints. *J. Bone Jt. Surg.* **5**, 569–578.

Sinelnikoff, E. & Grigorowitsch, M. (1931). Die Beweglichkeit der Gelenke als sekundäres geschlechtliches und konstitutionelles Merkmal. *Z. ges. Anat.* 2. *Z. KonstLehre*, **15**, 679–693.

West, C. C. (1945). Measurement of joint motion. *Arch. phys. Med.* **26**, 414–425.

Wiechec, F. J. & Krusen, F. H. (1939). A new method of joint measurement and a review of the literature. *Amer. J. Surg.* **43**, 659–668.

THE CENTRIFUGAL SPREAD OF STRUCTURAL CHANGE AT THE NODES IN DEGENERATING MAMMALIAN NERVES

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INTRODUCTION

During a histological study of the first changes in nerve degeneration it was found that the myelin retracts from the node at a very early stage (Causey & Palmer 1952). There were indications that this retraction of myelin at the nodes involved the nodes in an orderly sequence down the nerves from the crush to the periphery.

In view of the diversity of opinion as to the direction of onset of functional failure in a degenerating nerve (Rosenblueth & Dempsey, 1939; Rosenblueth & del Pozo, 1943; Parker, 1933; Titeca, 1935; Holobut & Jalowy, 1936; Erlanger & Schoepfle, 1946) and the possibility that increase in the naked axon area at the node might stop conduction in a myelinated fibre (Rushton 1951), we have made a careful investigation of the spread of this nodal involvement and have confirmed our previous findings.

METHOD

The nerve to the medial head of gastrocnemius (N.G.M.), and the phrenic nerve of rabbits were used.

With the rabbit under nembutal and ether anaesthesia, the N.G.M. was crushed in the upper part of the thigh with smooth-bladed watchmakers' forceps for 10 sec. After periods of 0–120 hr., the nerve was fixed *in situ* with a modified Flemming solution and embedded in paraffin and cut in serial sections at 5μ; staining was by the Weigert method. A more detailed description of the histological method may be found in Causey & Palmer (1952).

The phrenic nerve was crushed (in a few cases cut) in the neck. The site of the crush was marked by a loose cotton tie and at biopsy the whole nerve was excised. Pieces of the phrenic nerve 1 cm. in length (in this case in the fresh specimen) were fixed on cards in Flemming's solution and embedded as above. About 400 serial sections were cut from the middle of each centimetre piece.

The appearance of a normal node in successive serial sections at 5μ are shown in Pl. 1, figs. 1–4. The fibre marked '*A*' can be followed in the four successive sections; the narrowing of the axoplasmic canal is seen in figs. 2 and 3 but in none of these 5μ sections does the myelin fail to surround the axoplasm completely.

The method of fixation was kept constant throughout, and only four nodes were found in 160 normal nodes with absence of myelin in a 5μ section. This does not completely agree with the findings of Hess & Young (1952), who illustrate three nodes with a 5μ gap in the myelin at the node. Their method of fixation was similar to ours but they do not report on measurements of a large number of normal nodes. Young (1952), using fresh teased fibres, obtained a mean figure for the myelin free gap of $0{\cdot}5\mu$. The length of the naked axon at the node in well-fixed Flemming material is probably much less than 5μ.

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In a normal nerve, therefore, cut at 5μ thickness, there will be very rarely a section of a given node which is free from myelin. If, however, the naked part of the axon becomes larger than 5μ there will be sections through a node where no myelin is stained.

Pl. 1, figs. 5–8, show four successive sections from a fibre in a nerve that had degenerated for 72 hr. Fig. 6 shows the narrow axoplasmic canal surrounded by deeply stained myelin. In fig. 8, however, the myelin is very faintly and diffusely shown. Fig. 8 shows the enormous increase in stainable myelin only 5μ away.

In the serial sections of the N.G.M., nodes were examined and the number of sections without myelin staining was recorded. At least ten nodes taken at random were examined at each level, and the distance between the levels was calculated from the sections, so that the distances given in the results on the N.G.M. are of the nerve after fixation. With the phrenic nerve at least ten nodes from each piece of nerve were examined.

In the analysis of the data obtained, the hypothesis was set up that the length of axon free from myelin at the nodes varied as the time after crushing and the distance from the crush. That is to say,

$$y = ax_1 + bx_2 + c,$$

where y = length of axon free from myelin x_1 = time after crush (hr.), x_2 = distance from crush (mm.), and a, b and c are constants.

The evaluation of the constants a, b and c and their variance was carried out by Mr D. Sholl.

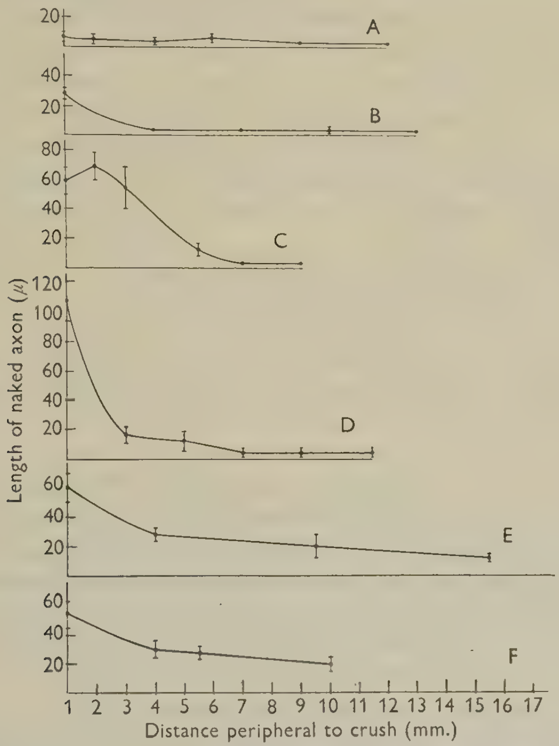
RESULTS

The appearance of the fibres at the neck level and diaphragmatic level of the phrenic nerve 72 and 96 hr. after a crush are shown in Pl. 2, figs. 10–13. It will be seen at the upper end of the nerve, just beyond the crush in both the 72 and 96 hr. specimens, that there are large distended fibres and that there are no normal nodes like those illustrated in Pl. 1, figs. 2 and 3. There are, of course, many nodes in different stages of retraction of the myelin but no node with the thick, crenated myelin and narrow axoplasmic canal. The sections from the distal ends of the specimens differ. The 96 hr. specimen, like the proximal ends of both specimens, shows no normal nodes and large distended fibres, but the distal end of the 72 hr. specimen shows normal nodes as '*n*', and is, in fact, indistinguishable from a transverse section of a normal phrenic nerve.

In examining these pictures, attention is drawn to the curious appearance of some of the fibres in the degenerating specimens such as '*s*', Pl. 2, figs. 10–13. The myelin is split and in-folded. This appearance has been observed regularly in mammalian nerves fixed with Flemming's solution when degeneration has gone on for 72 hr. or more. It has also been seen in 72 hr. degenerated fibres fixed in osmic acid and cut frozen, but not in formalin-fixed material. The effect seems to be due to rupture of distended parts of degenerating fibres when they harden in the fixative, and does not occur in normal fibres. Pl. 1, fig. 9, shows a longitudinal segment of a teased fibre showing the split in the length of the ovoid of myelin stained with osmic acid.

The evidence of the photographs is suggestive of a peripheral spread of disorganization of the myelin, but for quantitative analysis the measurement of a large number of nodes at different levels is necessary.

The graphs in Text-fig. 1 show the pooled results of measurements of 600 nodes in the nerve to the gastrocnemius muscle.



Text-fig. 1. Graphs of the N.G.M. in rabbit at various times after crushing. The height of the ordinate represents the unmyelinated length of axon at the node, from the crush towards the periphery, and each ordinate represents the mean of at least ten readings. The abscissa is measured in mm. from the crush. A, 0–1 hr.; B, 1–10 hr.; C, 30 hr.; D, 48 hr.; E, 72 hr.; F, 96 hr. The standard error of the mean is shown.

Text-fig. 1A shows the pooled results of measurements in nerves between 0 and 1 hr. after crush. Within 1 mm. of the crush there are a few fibres with measurable increase in the naked axonal length, but at 3, 5, 7, 11 mm. below the crush there is no increase in the length of axon at the node free from myelin.

Text-fig. 1B is from three nerves at 1–10 hr. after crush. The amount of naked axon has increased very markedly close to the crush, and at the 1 mm. level one gets five or six sections of an individual nerve without any myelin, so that there is as much as 30μ of axon which are free from any myelin.

Text-fig. 1C shows the pooled results round 30 hr. Close to the crush the number of sections free from myelin amounts to as many as twenty, giving a myelin-free gap of 100μ.

Text-fig. 1D, E and F show plots of six nerves at later stages after crushing, the nodal retraction of myelin at the more distal level increases in relation to time until at 96 hr. the length of naked axon at the node is about the same at all the levels along the nerve.

After longer periods of degeneration, such as the 96 hr. specimen Text-fig. 1F, the mean gap may be considerably less than in the earlier specimen. There is a much greater variation in the size of the gaps in these nerves, and one cannot be certain in these later stages that it is only nodal gaps which are being measured. The gaps produced by the breaking-up of the internodal lengths of myelin into ovoids cannot be distinguished from nodal gaps. Erzholz granules can sometimes be seen in this type of break, and the spacing of the gaps in individual fibres becomes irregular.

The tables of crude data are too large for inclusion in this paper, but complete copies have been deposited in the Thane Library of University College, London. The standard error of the mean has been indicated for the readings at each level plotted in the graphs.

The analysis of these figures gives the following result:

$$y = 35{\cdot}74 + 0{\cdot}09x_1 - 2{\cdot}78x_2,$$

the coefficients of both x_1 and x_2 are highly significant ($P < 0{\cdot}01$). The relation of this analysis to the analysis of the similar observations on the phrenic nerve is discussed below.

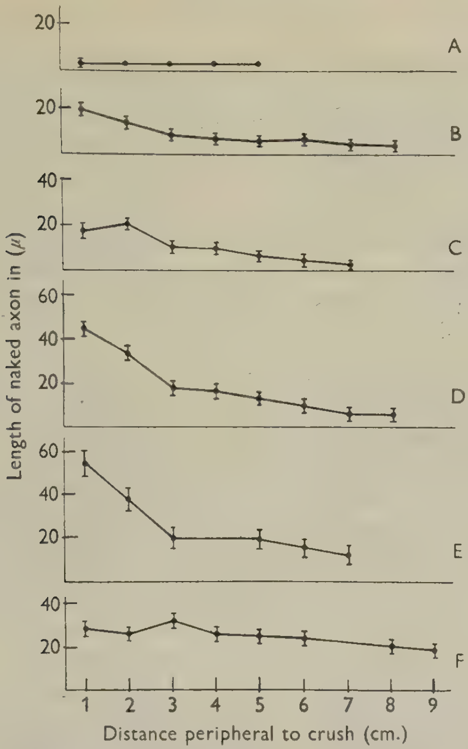
These measurements of the N.G.M. are over a short distance, and we have therefore examined the phrenic nerve to obtain a greater length of nerve. In dissecting out the phrenic nerve, a search was made for accessory branches entering the nerve in the upper part of the thorax which had been missed in the crush or cut in the neck. These were found on one occasion only, and in the resulting sections it was possible to trace a normal nerve bundle throughout the length of the nerve. This bundle has been disregarded in the subsequent measurement of nodes.

The normal phrenic nerve shows nodes all less than 5μ at five levels along an 8 cm. length of nerve. The pooled results are plotted in Text-fig. 2A.

The other plots in Text-fig. 2 show the distribution of retraction at 48, 60, 72 and 96 hr. after crushing; the steady progress of the involvement of nodes with increasing time of degeneration is clearly shown, until at 96 hr. the whole of the 8 cm. of nerve examined show long stretches of naked axon at the nodes amounting to some 50–60μ.

The four graphs B, C, D and F in Text-fig. 2 represent the pooled measurements of about 800 nodes, and the analysis of these figures gives the coefficient of x_1 as 0·35 and of x_2 as $-2{\cdot}63$, both of these are significant at $P < 0{\cdot}01$.

The analysis of the data on the phrenic nerves yields results similar to those found for the N.G.M. The hypothesis that the 'distraction' depends jointly on the time elapsed after the lesion and the 'distance', was tested by multiple regression analysis. The two regression coefficients were highly significant ($P < 0{\cdot}01$). This implies that after the lapse of a given time the 'distraction' decreases with the distance while, at a given distance, the distraction increases with the time after the lesion.



Text-fig. 2. Graphs of the phrenic nerve in rabbit at various times after crushing. The height of the ordinate represents the unmyelinated length of axon at the node, from the crush towards the periphery, and each ordinate represents the mean of at least ten readings. The abscissa is measured in cm. from the crush. A, normal nerve; B, 48 hr.; C, 53 hr.; D, 60 hr.; E, 72 hr.; F, 96 hr. The standard error of the mean is shown.

DISCUSSION

In considering the onset of failure of conduction in degenerating nerve fibres there is one aspect on which there is general agreement. Namely, that there is after a crush a latent period of about 48 hr., in mammalian nerve, during which conduction is 'normal'. In the histological examination of degenerating nerves the first changes are said to occur in the axon (Ramón y Cajal, 1928; Speidel, 1933; Weddell & Glees, 1941; Holobut & Jalowy, 1936), and these changes precede by many hours the onset of demonstrable failure of conduction. Erlanger & Schoepfle (1946), in view of this situation, wrote '...conduction ceases only with the disruption of the myelin sheath'. Whether actual disruption of the internodal myelin is a necessary precursor of failure of conduction has never been shown, and it seems more probable that if one accepts the saltatory nature of conduction in myelinated nerve fibres, then increase of the naked axon area at the node might in itself be sufficient to block conduction. According to Rushton (1951), the total nodal current is proportional to the axonal area at the node. It has been shown in these experiments that the naked axonal length at the node increases by a factor of more than ten times, before the break up of the internodal myelin column.

The observation that the retraction at the nodes proceeds after a latent period in a centrifugal direction, is of interest in indicating at least one structural change that has an orderly and not random progression. If the retraction at the nodes does in fact cause a conduction block, then it should be possible to obtain a nerve trunk in which at least some of the fibres had reached the stage of failure of conduction in the proximal part of the degenerating nerve, and not in the distal part. This possibility is being investigated.

As for the cause of peripheral progression of involvement of nodes, it is unlikely that this could be explained by the removal of a pressure exerted by the nerve cell along the length of the nerve fibre, because any loss of turgor pressure would be effective along the whole length of the fibre within a very short time, and it would be difficult to explain the delayed onset of the process.

It seems more likely that some substance or substances are being used up to maintain the form of the nerve—that this material is effective for some 48 hr., but with failure of replacement there is a final breakdown of the necessary metabolic processes. This view would not explain the sequence of involvement but only the delayed onset, and it is necessary on this hypothesis further to assume that either the current of injury or the local disorganization of the fine structure (see Causey & Palmer, 1952) act as a focal point, from which the changes spread in a manner analogous to the spread of crystallization in a supersaturated solution from the site of introduced crystal or foreign body.

SUMMARY

1. In the degeneration of myelinated mammalian nerve the myelin retracts from the node during the early stages of degeneration. The area of naked axon at the node is therefore increased.

2. The retraction first affects the nodes close to the lesion producing the degeneration and spreads in a peripheral direction.

We wish to thank Mr Sholl for the statistical analysis of the data. We thank Prof. J. Z. Young, F.R.S., for his interest throughout, and Mr J. Armstrong and Mr B. Whitton for photographic and technical assistance.

REFERENCES

Causey, G. & Palmer, E. (1952). Early changes in degenerating mammalian nerves. *Proc. Roy. Soc.* B, **139**, 597–609.

Erlanger, J. & Schoepfle, G. M. (1946). A study of nerve degeneration and regeneration. *Amer. J. Physiol.* **147**, 550–581.

Hess, A. & Young, J. Z. (1952). The nodes of Ranvier. *Proc. Roy. Soc.* B, **140**, 301–320.

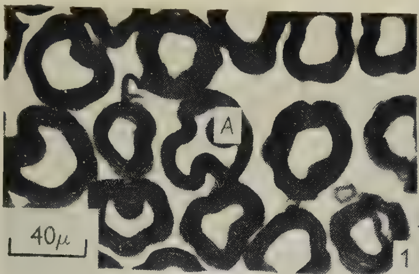
Holobut, W. S. & Jalowy, B. (1936). Die De- und Regeneration des peripheren motorischen Nervensystems auf einer morphologisch-funktionellen Grundlage. *Z. Zellforsch.* **25**, 541–564.

Parker, G. H. (1933). The progressive degeneration of frog nerve. *Amer. J. Physiol.* **106**, 398–403.

Ramón y Cajal, S. (1928). *Degeneration and Regeneration of the Nervous System.* Oxford: University Press.

Rosenblueth, A. & Dempsey, E. W. (1939). A study of Wallerian degeneration. *Amer. J. Physiol.* **128**, 19–30.

Rosenblueth, A. & del Pozo, E. C. (1943). The centrifugal course of Wallerian degeneration. *Amer. J. Physiol.* **139**, 247–254.



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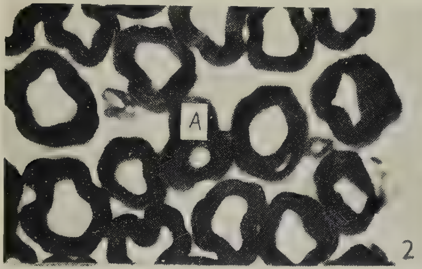

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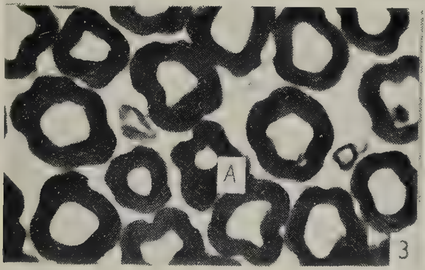

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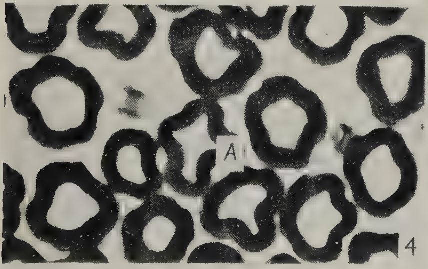

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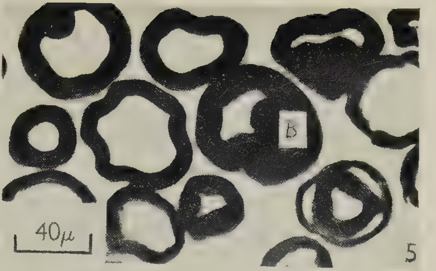

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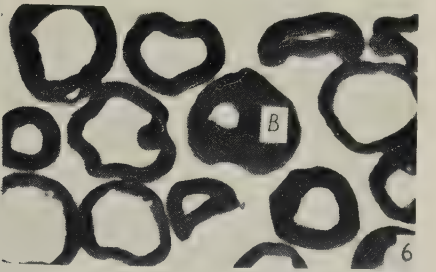

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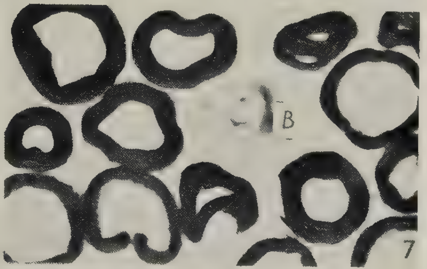

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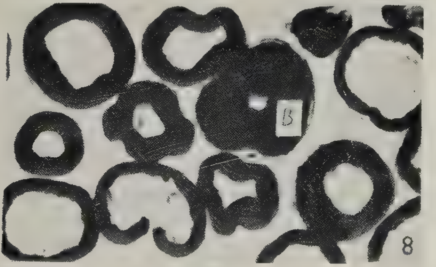

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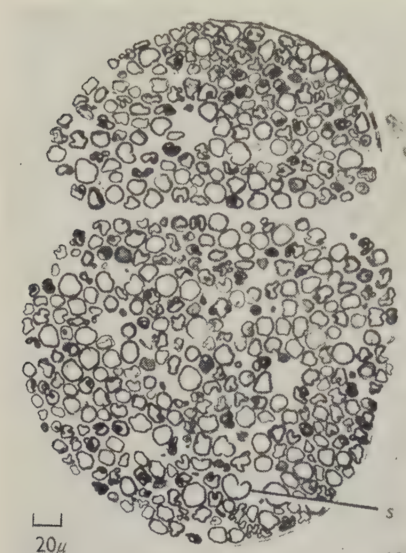
CAUSEY AND PALMER—STRUCTURAL CHANGE IN DEGENERATING MAMMALIAN NERVES



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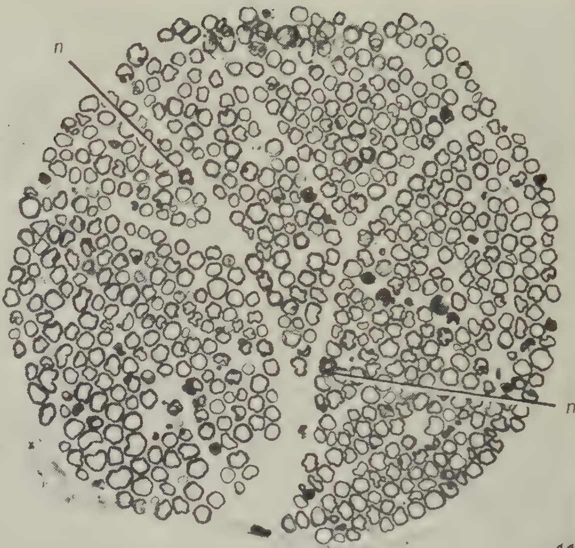

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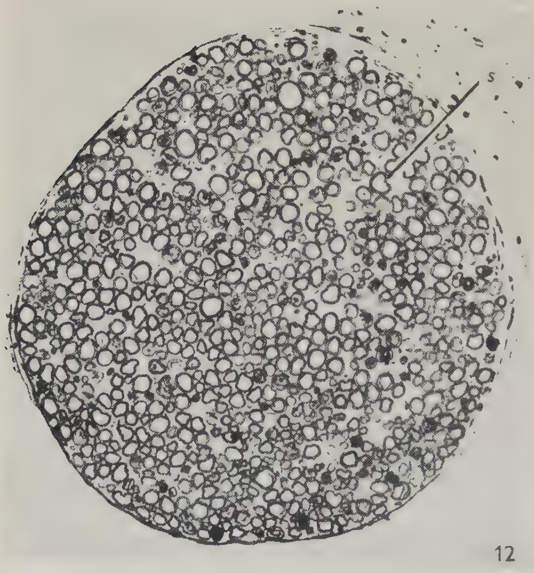

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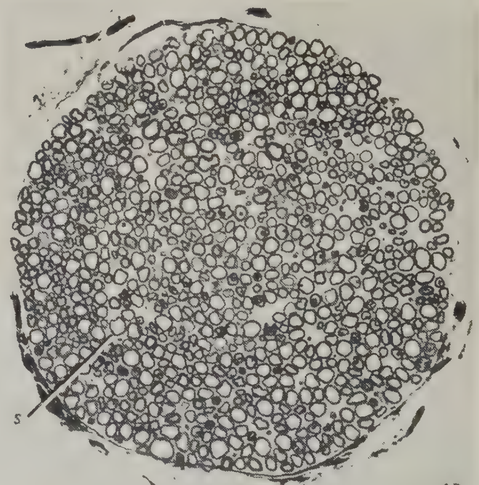

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CAUSEY AND PALMER—STRUCTURAL CHANGE IN DEGENERATING MAMMALIAN NERVES

RUSHTON, W. A. H. (1951). A theory of the effects of fibre size in medullated nerve. *J. Physiol.* **115**, 101–122.

SPEIDEL, C. C. (1933). Studies of living nerves. *Amer. J. Anat.* **52**, 1–79.

TITECA, J. (1935). Étude des modifications fonctionelles du nerf au cours de sa dégénérescence Wallérienne. *Arch. int. Physiol.* **41**, 1–56.

WEDDELL, G. & GLEES, P. (1941). The early stages in the degeneration of cutaneous nerve fibres *J. Anat., Lond.*, **76**, 65–93.

YOUNG, J. Z. (1952). Dimensions at the nodes of Ranvier. *J. Anat., Lond.*, **86**, 476.

EXPLANATION OF PLATES

All nerves are fixed in Flemming solution and stained with the Weigert-Pal technique, except in Pl. I, fig. 9. The scale is indicated in the figures.

PLATE 1

Figs. 1–4. Successive serial transverse sections of a normal phrenic nerve. A typical normal node is seen in fibre 'A'.

Figs. 5–8. Successive serial transverse sections of a phrenic nerve after 72 hr. degeneration. Abnormal node in fibre 'B'. Note the almost complete absence of myelin in fig. 7.

Fig. 9. Teased fibres of N.G.M. after 72 hr. degeneration. The myelin is showing retraction and Erzholz granules can be seen. The longitudinal line in the central fibre is probably due to the splitting of the myelin discussed in the text.

PLATE 2

Fig. 10. Section of phrenic nerve after 72 hr. degeneration from the neck region, taken immediately below the crush. Note enlarged fibres and split fibres '*s*'. No normal nodes.

Fig. 11. Section of the same nerve as in fig. 10 at the diaphragmatic level, 7 cm. below the crush. Note normal nodes '*n*'.

Fig. 12. Section of phrenic nerve after 96 hr. degeneration taken from the neck level immediately below the crush. No normal nodes, note split '*s*' and enlarged fibres.

Fig. 13. Section through the same nerve as in fig. 12 at the diaphragmatic level, taken 8 cm. below the crush. The section presents the same absence of normal nodes with split and enlarged fibres as the neck level.

THE EARLY DEVELOPMENT OF THE FEMORO-TIBIAL AND TIBIO-FIBULAR JOINTS

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Department of Anatomy, University of Sheffield

INTRODUCTION

A general survey of the development of the joints of the limbs (Haines, 1947) supported the views originally advanced by Hagen-Torn (1882) that the early continuous skeletal blastema gave rise to cartilages with their perichondria and to interzones, the interzones indicating for the first time the precise localities of future joint formation. A capsule developed around the joint region, cutting off the synovial mesenchyme within from the general mesenchyme without, and later the intracapsular ligaments were formed as condensations in the synovial mesenchyme. The interzones eventually became three-layered, the two outer denser layers being chondrogenic and forming articular cartilage, and the middle looser layer breaking down to form the central part of the joint cavity, while the more peripheral parts of the cavity were formed in the synovial mesenchyme.

Recently joint development has been carefully considered in the knee (Gray & Gardner, 1950; Gardner, 1950), hip (Gardner & Gray, 1950) and elbow (Gray & Gardner, 1951) with a wealth of new detail, particularly as regards the later stages of 'completion', after the appearance of the joint cavities. But while these authors agree in general with the views summarized above they mention two particular difficulties. In the knee a subdivision of an originally single femoro-tibial interzone into medial and lateral portions for the two condylar cavities was difficult to understand (Gray & Gardner, 1950, p. 277). They also found (p. 266) 'no indication of a fibrous capsule in the knee joints of specimens up to and including those 8½ weeks of age', that is until after the appearance of the cruciate ligaments. In other joints too, although they found the capsule present at much earlier stages, it was irregular in time of appearance and incomplete in extent (Gardner & Gray, 1950, pp. 181 and 186), being 'at first limited to those areas destined to be reinforced by extracapsular ligaments' (Gray & Gardner 1951, p. 450).

O'Rahilly (1951, p. 166) has reaffirmed the presence in human embryos of about 17–20 mm. of a femoro-fibular articulation, originally reported by Henke & Reyher (1874), with an interzone between the cartilages, and has concluded (p. 169) that there is no necessary association between an interzone in the embryo and a joint in the adult. These difficulties centre round the knee, and suggest that a re-examination of its early development, with particular attention to the soft parts, might throw new light on its construction. The comparable material described in the literature will be mentioned as the appropriate stages are discussed.

MATERIAL

The embryos chosen to illustrate the developmental succession are shown in Table 1. The knees of several embryos were reconstructed in cardboard, and the positions of most of the sections figured in the plates are indicated on the models. The internal organs such as the cartilages and nerves were modelled as solids, and in every tenth section the ectoderm was outlined, so that the drawings show the outlines of the limbs. It is true that the definition of 'the limits of the developing condensations depends in no small measure on personal interpretation' and that models may give 'a very misleading appearance of marginal definition' (O'Rahilly, 1951), but models do give detailed information as to spatial relationships that cannot be obtained otherwise.

Table 1

	C.R. length (mm.)		C.R. length (mm.)
Hamilton's 'Doyle'	12	Hamilton's 'Douglas'	18
Davies's 'No. 6'	13	Davies's 'No. 4'	20
Hamilton's 'Hall'	13	Middlesex 'Series 8'	21
Boyd's 'H 23'	13	Hamilton's embryo	22
Middlesex 'Series 1'	16	Davies's 'No. 5'	25
Hamilton's 'Futcher'	16	Boyd's 'H 11'	26
Davies's 'No. 1'	16	West's embryo	34

OBSERVATIONS

Appearance of the skeletal elements, 12 *mm*. The femur, tibia and fibula appear as separate chondrifications in the skeletal blastema of the limb. The earliest stages of development are illustrated by Hamilton's embryo 'Doyle' of 12 mm. and Davies's embryo 'No. 6' of 13 mm., with reconstructed femur lengths 0·6 and 0·8 mm., falling in Streeter's (1949, 1951) horizon early XVIII. In each specimen the femur has a well-defined head and shaft, but though the condylar region is indicated in blastemal tissue it is not yet chondrified, and so does not appear in Text-figs. 1 and 2 which show the chondrified parts but do not include the blastema. The tibia and fibula are found as rounded, somewhat elongated cartilages (Pl. 1, fig. 1), the tibia larger than the fibula, particularly in width, and on a more anterior plane. The axial artery crosses the distal part of the femur ventrally from the lateral to the medial part of the thigh, and then makes a sharp bend at the point where the femoral artery joins it later in development. It passes distally to the tibio-fibular interval, making a second bend and giving a pair of anterior branches, the more proximal of which probably represents the anterior tibial stem. It ends by giving a plexiform perforating tarsal artery to the dorsum of the foot and plantar branches to the plantar surface.

The region of the future knee joint is occupied by a mass of dense blastemal tissue which fills the space between the femur and tibia and fibula (Pl. 1, fig. 2), but the cartilages are remote from each other and no definite interzones can be distinguished; nor is it possible to define the boundaries of the skeletal blastema exactly, for the skeletal, perineural, perivascular and early muscular condensations are at this stage continuous with each other (Haines, 1947, p. 34). There is no indication of either the tibial condyles or the head of the fibula, and the fact that the fibula has not yet reached the level of the lateral popliteal nerve confirms the absence of the head at this stage. The greater part of the tibia lies proximal to the anterior tibial artery, suggesting that the proximal part of the cartilage is more advanced than the distal. The crowding together of the branches of the axis artery confirms the impression that the tibio-fibular region is relatively short, though its precise extent in the skeletal blastema cannot yet be marked off.

At the hip, shoulder and elbow of this embryo the cartilages have approached each other and interzones are found. Thus the knee in its degree of development corresponds to that of the elbow at an earlier stage, Streeter's (1949) horizon XVII.

In Fürst's (1925) 14 mm. embryo the cartilages are larger and the condyles of the femur have chondrified, but the relative positions and uncomplicated shapes of the tibia and fibula are similar, and Gray & Gardner (1950) describe a similar state at 12 mm. The blood vessels agree well with those of Senior's (1919) 12 mm. embryo, and he also found the tibio-fibular interspace very short.

In Hamilton's 13 mm. embryo 'Hall', reconstructed femur length 0·95 mm., the tibial head has expanded, so that the tibia as a whole is wedge-shaped, but it is still remote from the as yet unexpanded lower end of the femur (Text-fig. 3). The blood vessels, which are well preserved, confirm two observations made by Senior (1919, 1920). The anterior tibial artery takes a strongly recurrent course as it passes towards the anterior tibial nerve, suggesting that the first of the adult branches to become recognizable is the anterior tibial recurrent artery. The axial artery gives off, at the sharp bend it makes after passing behind the lower end of the femur, the ramus communicans superius which continues the direction of the proximal limb of the angulation towards the medial aspect of the thigh, and then turns proximally to end in the capillary plexus of the adductor muscle mass (Pl. 1, fig. 3). The plexus is, at this stage, the only connexion between it and the femoral artery.

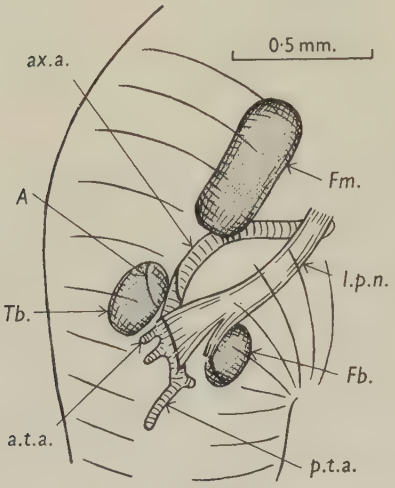


Fig. 1.

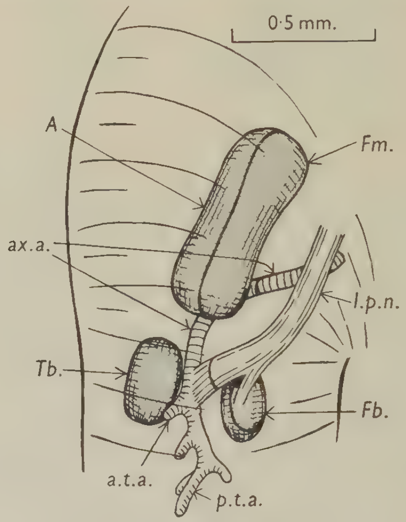


Fig. 2.

Text-fig. 1. Hamilton's 12 mm. embryo 'Doyle'. Early chondrification of the tibia and fibula. For section *A* see Pl. 1, fig. 1.

Text-fig. 2. Davies's 13 mm. embryo 'No. 6'. Ends of the cartilages still unexpanded and remote from each other. For section *A* see Pl. 1, fig. 2.

Interzone formation. In Boyd's 13 mm. embryo, H 23, reconstructed femur length 0·95 mm., the lower end of the femur is somewhat widened to form a condylar region (Text-fig. 4). The fibula has elongated and is placed under the overhanging lateral femoral condyle, but has not yet reached the level of the lateral popliteal nerve, and is still remote from the femur. The intervening space is filled by a homogeneous mass of condensed tissue. The tibia is now a wedge-shaped cartilage with a widened head and a distinct shaft. The lateral condylar region of the tibia does not yet, however, intervene between any part of the fibula and the lateral femoral condyle, nor does it make a close approach to the femur. In the intercondylar region a mass of dense tissue again separates the tibia from the femur, but in the region of the medial condyles, though the femoral condyle is poorly chondrified, the tissue between the cartilages is sufficiently narrowed to allow the distinction of an interzone (Pl. 1, fig. 4; cf. Haines, 1947, fig. 10 for a general low-power view).

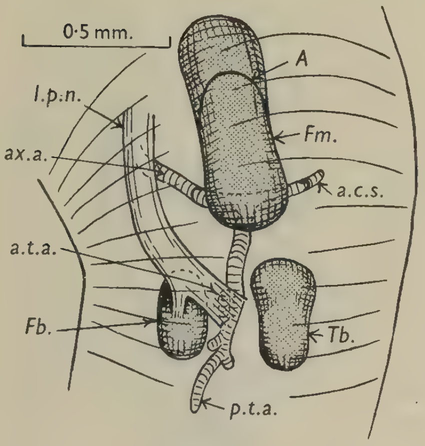


Fig.3.

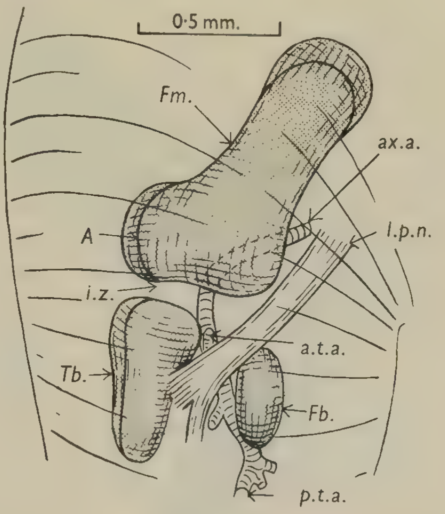


Fig. 4.

Text-fig. 3. Hamilton's 13 mm. embryo 'Hall'. Anterior tibial artery distinct. For section *A* see Pl. 1, fig. 3.

Text-fig. 4. Boyd's 13 mm. embryo 'H 23'. Tibia and femur now close together, separated by an interzone. For section *A* see Pl. 1, fig. 4.

The appearances suggest that the medial condyle of the tibia comes into close relation with the femur before the lateral, but the outlines of the tissues are, at this stage, still difficult to place precisely. The general development agrees well with Bardeen's (1905) embryo CIX, 11 mm., where the lower end of the femur and upper end of the tibia are expanded while the fibula is remote from both. Bardeen's CLXXV, 13 mm., is more advanced, for there is cartilage in the metatarsal region and a well-developed interzone at the knee.

Appearance of the capsule, and isolation of the fibula, 16 mm. In the Middlesex Hospital embryo 'Series 1', 16 mm., reconstructed femur length 1·4 mm., the knee is more sharply flexed than in the earlier stages. The condyles of the femur are very wide, and overhang both tibia and fibula (Text-fig. 5). The fibula is now more elongated but its upper end is still separated from the lateral popliteal nerve by a wide interval, partly filled by the thick perichondrium and partly by general mesenchyme, and the nerve is as close to the lateral condyle of the femur as it is to the upper end of the fibula. The lateral condyle of the tibia is still poorly developed, and projects feebly as compared with the lateral condyle of the femur, with which it makes only a small area of close association. It does not intervene between any part of the fibula and the femur, nor does it overhang the anterior tibial artery, which is now a well-defined trunk from the popliteal artery to the proximal surface of the anterior tibial continuation of the lateral popliteal nerve. The popliteal artery itself is derived from the union of the original axial artery, which still persists, with the femoral artery which has now joined it.

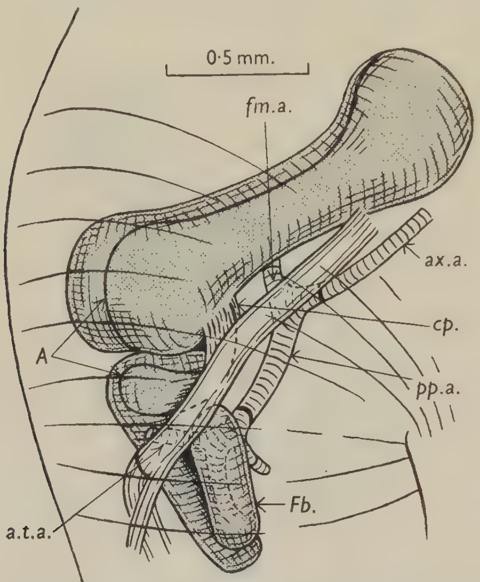


Fig. 5.

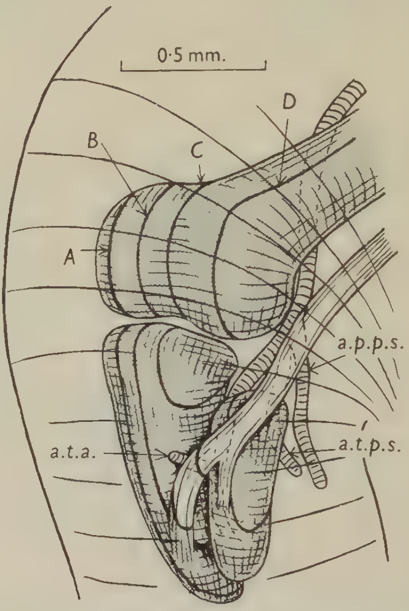


Fig. 6.

Text-fig. 5. Middlesex 16 mm. embryo 'series 1'. Appearance of capsule posterior to femoro-tibial joint. For section *A* see Pl. 1, fig. 5.

Text-fig. 6. Davies's 16 mm. embryo 'No. 1'. Medial and lateral condylar joints well defined. For sections *A–D* see Pl. 1, figs. 6–10.

A capsule has formed enclosing the condylar and intercondylar parts of the femur and tibia (Pl. 1, fig. 5; cf. Haines, 1947, fig. 16, for another section from this embryo). In section it appears as a band of longitudinally orientated cells joining the perichondria of the femur and tibia. It is convex posteriorly where loose mesenchyme separates it from the popliteal artery, and concave anteriorly where it faces the intercondylar mesenchyme, in which the cruciate ligaments have not yet developed. It does not include the fibula, but fades out into the rather dense tissue surrounding the lateral condyle of the femur.

The blood vessels agree with those of Senior's (1919) 14 mm. example, where the axial artery persisted though already joined by the femoral artery through the ramus communicans superior. Bardeen's (1905) CXLIV, 14 mm., shows the overhanging lateral femoral condyle, the relatively unexpanded tibia, and the remoteness of the fibula from both these cartilages.

Hamilton's 16 mm. embryo 'Futcher' is cut so that the plane of section passes between the femur and tibia cutting the interzones of both condylar joints. The plane makes reconstruction difficult, but the arrangement of the joint elements is diagrammatically clear (Pl. 1, fig. 6). Between the two interzones is a mass of intercondylar vascular mesenchyme. The blood vessels are not visible in the uncoloured photograph but the position of one of them is indicated in the figure. The intercondylar mesenchyme is separated posteriorly by the capsule from the general mesenchyme and axial artery. Anteriorly the intercondylar mesenchyme is continuous with that surrounding the patellar tendon. Gray & Gardner (1950) noted an intercondylar fossa in their 15 mm. specimens.

In Davies's embryo 'No 1' of 16 mm., measured after fixation, reconstructed femur length 1·2 mm., of Streeter's horizon XX, the lateral condyle of the tibia overhangs the anterior tibial artery and approaches more closely to the fibula, though it does not yet intervene between the femur and fibula, and still makes only a small area of apposition with the bulging lateral femoral condyle (Text-fig. 6). A section through the medial condylar region (Pl. 1, fig. 7) shows the slightly convex femoral condyle separated from the slightly concave tibial condyle by a plate of evenly dense blastemal tissue, a typical interzone, continuous at its periphery with the intercapsular parts of the perichondria of the two cartilages. The capsule again appears as a broad band of elongated cells passing between the tibia and femur. Posteriorly it is outlined by the general mesenchyme which separates it from the medial head of the gastrocnemius and anteriorly by the synovial mesenchyme which separates it from the intracapsular parts of the perichondria and the interzone.

A section passing through the intercondylar region (Pl. 1, fig. 8) shows a wider separation of the cartilages with a thick mass of undifferentiated tissue intervening. There is some indication of orientation of the cells of this tissue, but no definite cruciate ligaments can yet be distinguished. A section through the lateral condylar region (Pl. 1, fig. 9) shows again a typical evenly dense interzone, separated by the capsule from the popliteal artery which passes distally close to the tibia, between the cartilage and the popliteus muscle. Whereas in the 13 mm. embryo a homogeneous mass of dense tissue filled the space between the femur and the fibula, a section through the fibular region (Pl. 1, fig. 10) shows now a wide space intervening between, filled in equal proportion by the perichondria of the two cartilages and the undifferentiated mesenchyme between them. A search failed to detect any interzone between the tibia and the fibula, for their perichondria were separated throughout by loose mesenchymal tissue.

In Hamilton's 18 mm. embryo 'Douglas' the plane of section is sagittal to the embryo as a whole, but cuts the knee in what would correspond, in the adult, to the coronal plane. The lateral condyle of the tibia is now well developed, and has expanded above the anterior tibial artery to approach the fibula, but the two cartilages do not come into close opposition and are separated by loose mesenchyme (Text-fig. 7). The capsule, cut tangentially in the section (Pl. 1, fig. 11), passes from the femur to the tibia but has no attachment to the fibula. Two sharply defined cords attach the fibula to the femur, the fibular collateral ligament and the tendon of the popliteus. The ligament is bowed outwards, and the lateral popliteal nerve crosses its superficial surface (Pl. 1, fig. 12), while the tendon is attached to the head of the fibula before expanding into the muscle belly. The condensation of the fibular collateral ligament was noted by Gray & Gardner (1950, p. 277). In both this and Davies's 'No. 1' embryo, the femoral artery supplies all the blood to the popliteal artery, the old channel passing down the axial artery having been abandoned, but the popliteal artery still passes anterior to the popliteus.

In Davies's embryo 'No. 4', of Streeter's horizon XX, 20 mm. before fixation, the upper end of the tibia has expanded, especially laterally, where it now makes a wide contact with the lateral condyle of the femur, intervening between it and the upper end of the fibula (Text-fig. 8). The condyles of the femur and tibia are still united by dense interzones (Pl. 2, fig. 13), and the fibula makes no contact with either femur or tibia, for it is separated from both by a distinct layer of loose mesenchymal tissue (Pl. 2, figs. 15, 16).

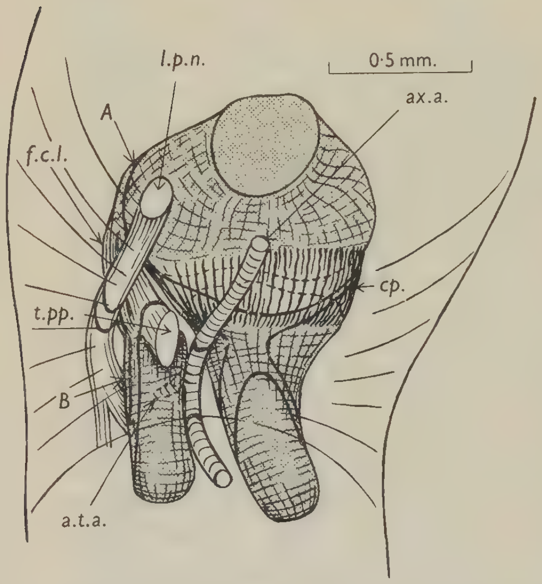


Fig. 7.

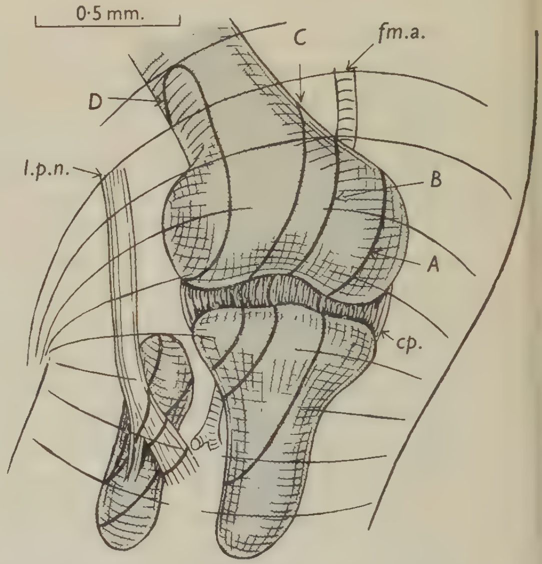


Fig. 8.

Text-fig. 7. Hamilton's 17 mm. embryo 'Douglas'. Fibular collateral ligament and tendon of popliteus defined. For sections *A* and *B* see Pl. 1, figs. 11 and 12.

Text-fig. 8. Davies's 20 mm. embryo 'No. 4'. Capsule still continuous posterior to knee. For sections *A–D* see Pl. 2, figs. 13–16.

In the intercondylar region (Pl. 2, fig. 14) the capsule appears as a thick sheet, separating the general mesenchyme of the popliteal fossa containing the popliteal artery from the synovial mesenchyme in which the cruciate ligaments are forming. The capsule has characteristically elongated cells, and is still bulged posteriorly, though the convexity is not so marked as before. Small vessels pierce the capsule to supply the intercondylar region.

These 18 and 20 mm. specimens agree well with Bardeen's (1905) models of embryos XVII, 18 mm., and XXII, 20 mm., and with Hesser's (1926) 22 mm., and Langer's (1929), 24 mm., models, but none of these show the capsule. A coronal section of a slightly older embryo, the Middlesex 21 mm. embryo 'Series 8', shows the medial collateral ligament, which at this stage resembles the capsule in structure and arrangement (Pl. 2, fig. 17).

Interruption of the capsule, 22–26 *mm.* In Hamilton's 22 mm. embryo the capsule, heretofore continuous throughout the posterior condylar and intercondylar regions, is broken (Text-fig. 9). Still persistent between either condyle (Pl. 2, fig. 18) it can no longer be distinguished opposite the cruciate ligaments which are now clearly defined, particularly the posterior member of the pair (Pl. 2, fig. 19). Thus the cruciate ligaments and axial artery are separated by loose mesenchyme only. In this mesenchyme blood vessels enter the interior of the joint.

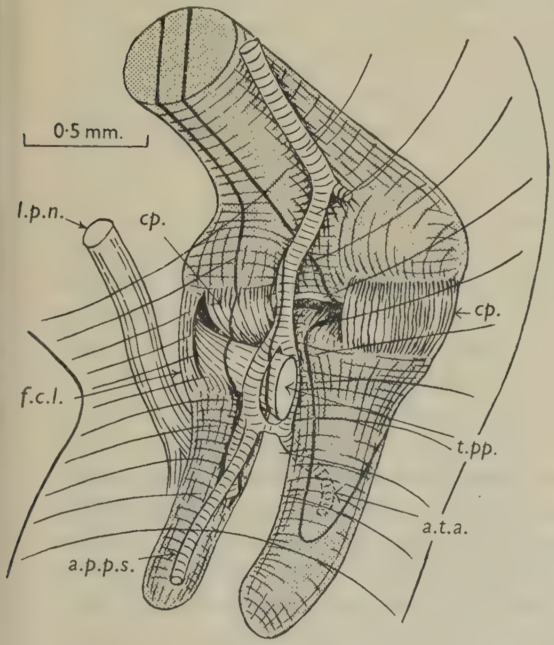


Fig. 9.

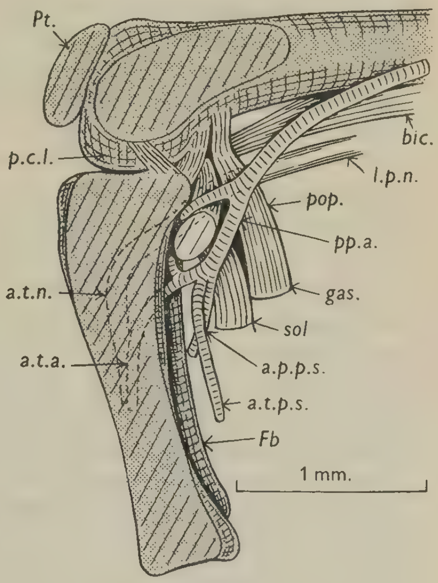


Fig. 10.

Text-fig. 9. Hamilton's 22 mm. embryo. Capsule now broken in intercondylar region. For sections *A* and *B* see Pl. 2, figs. 18 and 19.

Text-fig. 10. Boyd's 26 mm. embryo 'H 11'. Capsule still broken. For sections *A* and *B* see Pl. 2, figs. 20 and 21.

The popliteal artery now divides as it approaches the popliteus into two branches of equal calibre, Senior's (1919) arteria poplitea superficialis and arteria poplitea profunda, and these are connected distally to the popliteus so as to form a complete arterial circle round the muscle. The reconstruction agrees closely with Senior's 22 mm. specimen.

FORMATION OF THE TIBIO-FIBULAR INTERZONE (22 mm.)

In Boyd's 26 mm. embryo 'H 11' the lateral condyle of the tibia overhangs the greater part of the fibula (Text-fig. 10), and for the first time, a tibio-fibular interzone is found (Pl. 2, fig. 20). This is formed of a dense mass of tissue similar to that forming the femoro-tibial interzone, but still shows indications of its origin in the thin light zone between the two original perichondria. Gray & Gardner (1950) found a 'somewhat definite interzone' here at 22–25 mm., and cavity formation from about 75 mm. onwards.

The capsule in this embryo can be distinguished in the condylar regions (Pl. 2, fig. 20), where it is distinct from the popliteus and from the lateral head of the gastrocnemius, and it is very clear in Davies's 25 mm. embryo 'No. 5' (Pl. 2, fig. 22), separating the indefinite condensation for the medial meniscus from the tissues of the popliteal fossa and the popliteus muscles, but in both these embryos the capsule is indistinct in the intercondylar region (Pl. 2, figs. 21, 23). Langer (1929, p. 87) found no capsule posterior to the joint at 26 mm. but modelled it at 28 mm. finding it complete posterior to each condyle, but defective in the intercondylar region (p. 90). He did not find the capsule complete in the intercondylar region before 75 mm., but in West's 34 mm. embryo (Pl. 2, fig. 24) the intercondylar part of the capsule is again distinct, though faint, and this is the stage at which Gray & Gardner (1950) describe its first appearance.

DISCUSSION

The cartilages near the knee appear at about 13 mm. and by the 16 mm. stage there are two distinct interzones between the femur and tibia, one for each condylar region, while the intercondylar region is occupied by a looser tissue, presumably a general mesenchyme, in which the cruciate ligaments develop later. There is no evidence that the intercondylar tissue has migrated into the fossa between the condyles, for it appears *in situ* as the cartilages develop, separating the two interzones. Perforations of the skeletal blastema by general tissue certainly occur in the forearm and shin, between the radius and ulna and between the tibia and fibula, whose perichondria are separate at the time of their first formation (Bardeen, 1905). There is then no reason why the intercondylar tissue should not be a general mesenchyme rather than a skeletal blastema, though before the 16 mm. stage the tissues can hardly be distinguished histologically. As Gray & Gardner (1950, p. 277) have suggested, it is difficult to accept the subdivision of a single femoro-tibial interzone into two, but if there are two interzones from the time of their first appearance this difficulty does not arise.

It has long been recognized that the two condylar cavities of the knee are separate in early development, and only come to communicate with each other later, and Langer (1929) has prepared models illustrating these cavities. They remain separate, or nearly so, in several animals, including monotremes and ungulates, and it has been claimed that complete separation is the primitive condition in tetrapods, though in reptiles there is but one cavity (Nauck, 1938; Haines, 1942). The suggestion that there are two separate interzones from the time of their first appearance, but that at that time the tissues are too dense to distinguish from each other, throws the separation back to an earlier stage, but leaves open the question as to whether the separate cavities of those animals which possess them are truly primitive or merely neotenic.

Gardner & Gray (1950) emphatically denied the presence of the capsule in the early knee at 16–20 mm., though they and other authors have confirmed its presence in the shoulder (Lewis, 1902, 20 mm.; Hesser, 1926, 22 mm.; Haines, 1947, 21 mm.), elbow (Lewis, 1902, and Haines, 1947, 16 mm.; Gray & Gardner, 1951, 17 mm.), wrist (Haines, 1947, 19 mm.) and hip (Gardner & Gray, 1950, 20 mm.). In all these joints it appears as a thick bulging sheet of dense tissue passing between the perichondria of the skeletal elements visible in sagittal, transverse and coronal sections (Pl. 1, figs. 5, 6 and 11). In later embryonic and in adult stages the capsules may be poorly developed or deficient in certain areas—for example, at the posterior part of the elbow (Gray & Gardner, 1951, fig. 7, 30 mm.) or over the intercondylar region of the knee. But at the time of their first appearance they seem constant in position and consistence, and are not 'at first limited to those areas destined to be reinforced by extracapsular ligaments' (Gray & Gardner, 1951, p. 450). They are, in fact, found in both the positions mentioned, and at the elbow at first surround the humero-radial and humero-ulnar joints independently, the part destined to persist as the annular ligament being no more dense than that destined to be destroyed later as the two joints join (Pl. 2, fig. 25).

Gray & Gardner (1950, p. 278) have pointed out that the presence or absence of a capsule is a question independent of the main issue—whether the intracapsular structures are derived from the general mesenchyme as suggested by Hagen-Torn (1882) and Haines (1947), as they themselves believe, or from the blastema as suggested by other authors—but their denial of the existence of a capsule before the 30 mm. stage at the knee cannot be substantiated.

It has been suggested that the capsule is a blastemal formation, a continuation of the perichondria over the region of the joint (Baer, 1837, p. 96; Bardeen, 1905, p. 289). This cannot be entirely true, for before the capsule has appeared the embryonic perichondria can be traced into continuity with the edges of the interzones, and after its appearance it subdivides the perichondria into extra- and intracapsular parts. It is possible, however, that the capsule is formed in association with the outer fibrous layer of the perichondrium as part of a continuous condensation round the skeletal and joint tissues, together with the condensations for the muscle tendons and intermuscular septa.

The complete separation of the upper end of the fibula from both femur and tibia at 18–20 mm. is unexpected, and has not been reported previously. In earlier stages it is difficult to define the tissues in this region owing to their density, but at 18 mm. loose mesenchymal tissue surrounds all aspects of the head. The separation of the fibula from its primitive reptilian articulation with the tibia, so that the fibula and tibia come, in eutherian mammals, to rotate together as a unit, are well understood (Haines, 1942, p. 296). The mammalian embryonic phase in which the fibula articulates with neither femur nor tibia is a peculiar intermediate condition but does not resemble the phylogenetically intermediate stage, represented by the living monotremes and marsupials, in which the fibula articulates with both. The early attachment of the popliteus to the head of the fibula may, however, suggest the condition in reptiles, for there the tibia and fibula rotate independently and the popliteus passes between them.

The secondary formation of an interzone from the perichondria of two skeletal elements originally separated from each other by general mesenchyme is known in the patello-femoral joint. There, however, the interzone is formed later, at about

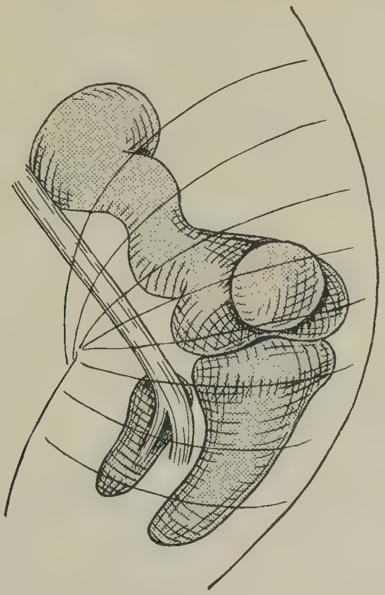
32 mm., and like the femoro-tibial interzones at that stage is definitely three-layered, for it never passes through a dense homogeneous stage (Haines, 1947, p. 42). The tibio-fibular interzone appearing by 26 mm. also resembles those of the main cavities of the knee at that stage, when they are still dense, though there is some lightening of the interior to initiate the three-layered stage.

O'Rahilly's (1951) recent reaffirmation of the existence of a femoro-fibular articulation in the early human embryo is based partly on specimens described in the literature by Henke & Reyher (1874), Bardeen (1905) and Fürst (1925), and partly on embryos he himself described. Henke & Reyher's section of an embryo of about 18 mm. and Fürst's model of a 20 mm. specimen show an upgrowth of the fibula coming into relation with the lateral condyle of the femur as does the fibular extension in *Echidna.* But Henke & Reyher found (p. 231) the shafts of the femur and fibula bent, and Retterer (1902) has remarked on the poor fixation of their material, while Fürst's model shows a tibia with a thick shaft and no condyles, out of apposition with the femur except in one small area, and these specimens must be regarded as deformed. Bardeen's fig. 5, of a model from a 14 mm. embryo, redrawn in outline by O'Rahilly, does appear to show the fibula close to the femur, but Bardeen's fig. 6, another view of the same model, shows that the cartilages were in fact far apart.

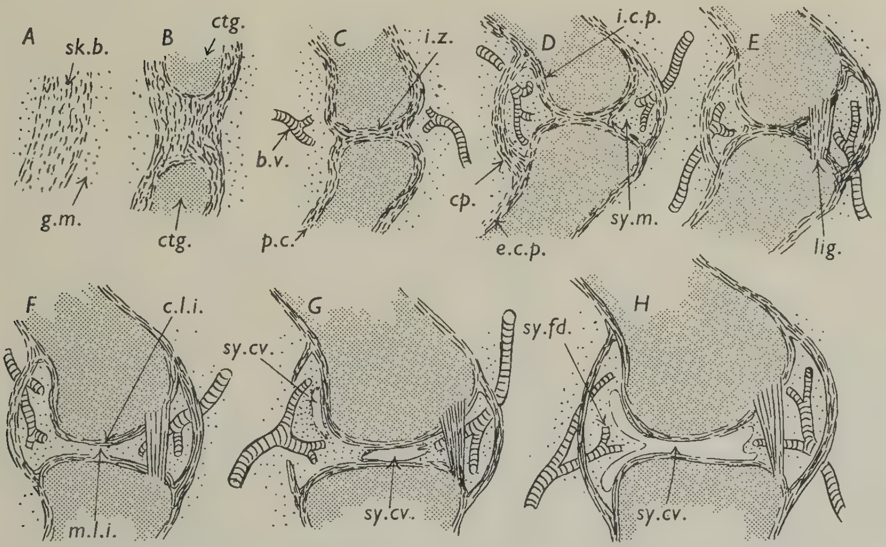
Davies's 16 mm. embryo 'No. 1' (Text-fig. 6 and Pl. 1, fig. 11) was described by O'Rahilly (1951, p. 166) as showing, between the femur and fibula, a 'three-layered interzone', consisting of 'two chondrogenic layers and a thin, looser, middle layer', but re-examination of the material shows that there are in fact two perichondria, one belonging to the femur and the other to the fibula, and undifferentiated tissue between. Three-layered interzones are not found at all in such early embryos, the earliest known being at the elbow in a 21 mm. specimen, and at the knee they are not found till the 26 mm. stage. Nor does the tissue filling the femoro-fibular interval resemble a three-layered interzone in structure, for the middle layer of the interzone is thin as compared with the chondrogenic zones (Haines, 1947, fig. 25).

O'Rahilly's 18 mm. embryo showing a fibular extension at the level of, or even exceeding the level of, the tibia, shows also a wide femoro-tibial apposition unknown in other embryos at that stage. His '19 mm.' embryo is too shrunken and deformed for use as critical evidence, for a reconstruction from the other side of this embryo shows the femur bent into an S shape (Text-fig. 11), and the presence of a well-chrondrified patella suggests a true length of at least 23 mm. Bardeen (1905), Langer (1929) and Gray & Gardner (1950) specifically deny the occurrence of a femoro-fibular articulation at any stage, and the present work supports this conclusion.

The findings may be summarized by a series of diagrams (Text-fig. 12) representing in simplified form the development of an ordinary limb joint. The blastema (*A*) is chondrified (*B*) from a number of centres and these approach each other to define the interzones (*C*) continuous at their edges with the perichondria. A capsule forms round the joint region cutting off the synovial mesenchyme and intracapsular parts of the perichondria (*D*). Condensations in the synovial mesenchyme give rise to the intracapsular ligaments (*E*). The interzone becomes three-layered (*F*), and dehisces (*G*), and the cavities spread into the synovial mesenchyme whose remains form the synovial and subsynovial tissues (*H*). The condylar cavities of the knee follow this general sequence of development.



Text-fig. 11. Davies's embryo 'No. 5'. Gross deformation.



Text-fig. 12. Diagrams illustrating the development of a typical synovial joint.

SUMMARY

1. A detailed account of the changing relationships of the cartilages and interzones of the knee is given from the time of their first appearance to the stage just preceding cavitation.

2. The interzones for the medial and lateral condylar regions are separate from the time of their first appearance, and are not formed by the subdivision of one originally single interzone.
3. The presence of a capsule posteriorly is confirmed in the earlier stages, though later it becomes deficient in the intercondylar region.
4. Before chondrification the fibular and femoral regions of the skeletal blastema are continuous, but soon after chondrification the fibula is found to be unattached to either the femur or the tibia.
5. The fibula never articulates with the femur at any stage, but eventually forms an interzone with the tibia.
6. A general scheme of joint development, with which the findings for the knee are consistent, is presented.

My thanks are due to Profs. Baxter, Boyd, F. Davies, Hamilton and Walls for the loan of material, and to Mr J. Kugler for technical assistance in preparation and photography.

REFERENCES

Baer, K. E. v. (1837). *Über Entwickelungsgeschichte der Thiere.* II Th. Königsberg: Bornträger.

Bardeen, C. R. (1905). Studies of the development of the human skeleton. *Amer. J. Anat.* 4, 265–302.

Fürst, C. M. (1925). Über die Entwicklung der Fibula beim Menschen. *Z. ges Anat.* 1. *Z. Anat. Entw-Gesch.* **76**, 113–116.

Gardner, E. (1950). Physiology of movable joints. *Physiol. Rev.* **30**, 127–176.

Gardner, E. & Gray, D. J. (1950). Prenatal development of the human hip joint. *Amer. J. Anat.* **87**, 163–212.

Gray, D. J. & Gardner, E. (1950). Prenatal development of the human knee and superior tibiofibular joints. *Amer. J. Anat.* **86**, 235–288.

Gray, D. J. & Gardner, E. (1951). Prenatal development of the human elbow joint. *Amer. J. Anat.* **88**, 429–470.

Hagen-Torn, O. (1882). Entwickelung und Bau der Synovialmembranen. *Arch. mikr. Anat.* **21**, 591–663.

Haines, R. W. (1942). The tetrapod knee joint. *J. Anat., Lond.*, **76**, 270–301.

Haines, R. W. (1947). The development of joints. *J. Anat., Lond.*, **81**, 33–55.

Henke, W. & Reyher, C. (1874). Studien über die Entwickelung der Extremitäten des Menschen inbesondere der Gelenkflächen. *S.B. Akad. Wiss Wien*, Abt. III. **70**, 217–273.

Hesser, C. (1926). Beitrag zur Kenntnis der Gelenkentwicklung beim Menschen. *Morph. Jb.* **55**, 489–567.

Langer, M. (1929). Über die Entwicklung des Kniegelenkes. *Z. ges. Anat.* 1. *Z. Anat. Entw-Gesch.* **89**, 83–101.

Lewis, W. H. (1902). The development of the arm in man. *Amer. J. Anat.* **1**, 145–184.

Nauck, E. T. (1938). Extremitätenskelett der Tetrapoden. In Bolk, L., Göppert, E., Kallius E., & Lubosch, W. *Handbuch der vergleichenden Anatomie der Wirbeltiere*, **5**, 71–248. Berlin and Wien: Urban and Schwarzenberg.

O'Rahilly, R. (1951). The early prenatal development of the human knee joint. *J. Anat., Lond.*, **85**, 166–170.

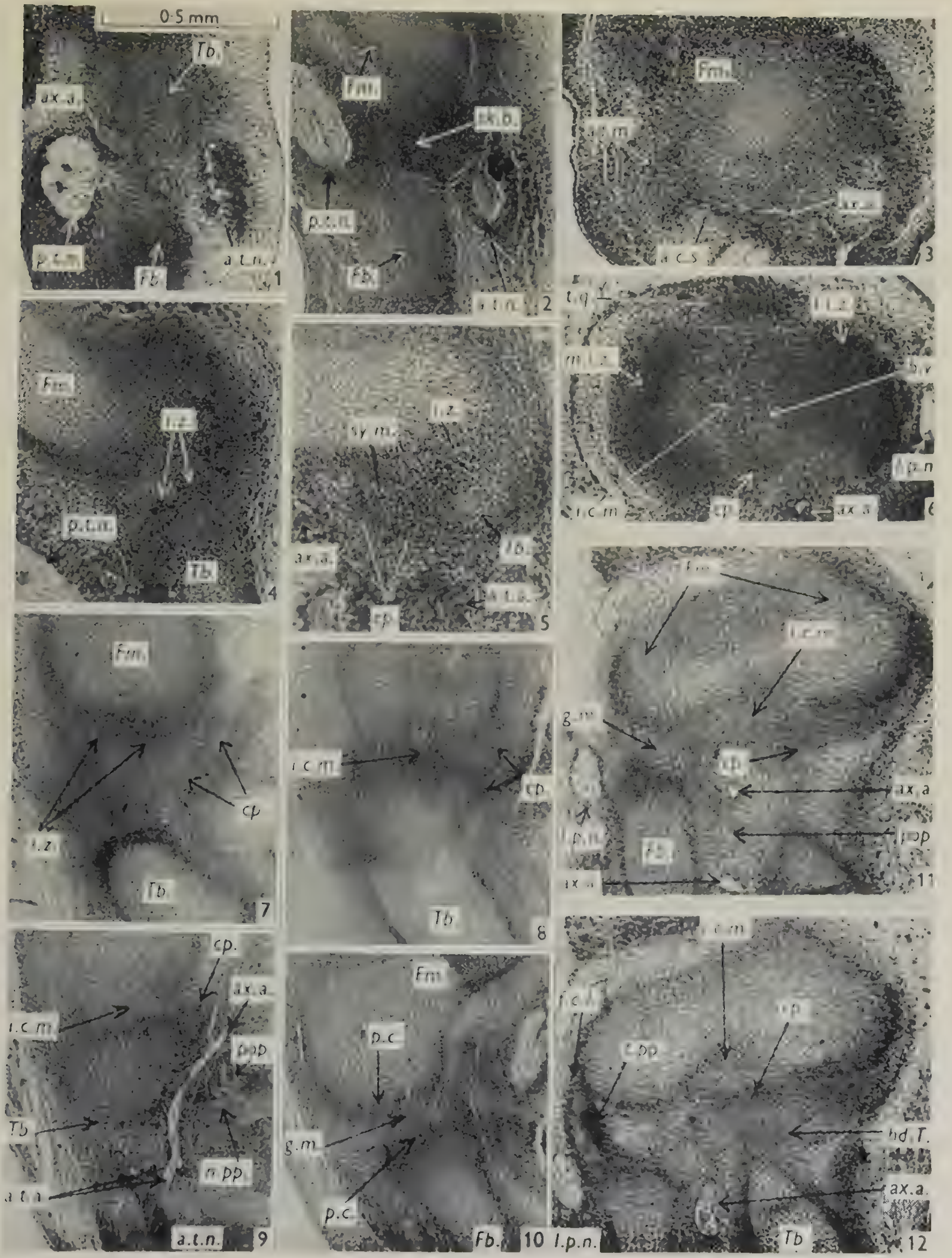
Retterer, E. (1902). Ébauche squelettogène des membres et développement des articulations. *J. Anat., Paris*, **38**, 473–509, 580–623.

Senior, H. D. (1919). The development of the arteries of the human lower extremity. *Amer. J. Anat.* **25**, 55–96.

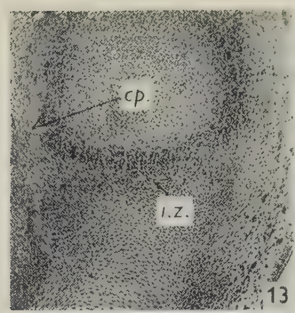
Senior, H. D. (1920). The development of the human femoral artery, a correction. *Anat. Rec.* **17**, 271–279.

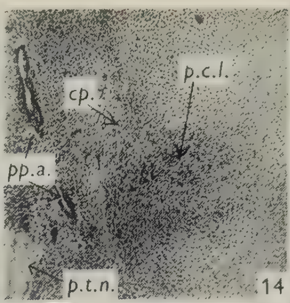
Streeter, G. L. (1949). Developmental horizons in human embryos (fourth issue). A review of the histogenesis of cartilage and bone. *Contr. Embryol. Carneg. Instn*, **33**, 149–167.

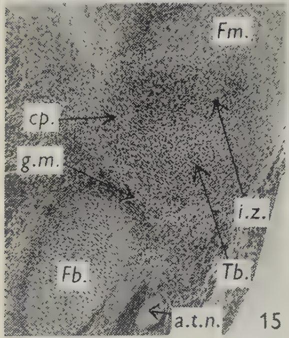
Streeter, G. L. (1951). Developmental horizons in human embryos (fifth issue). Descriptions of age groups xix, xx, xxi, xxii, and xxxiii. *Contr. Embryol. Carneg. Instn*, **34**, 165–196.

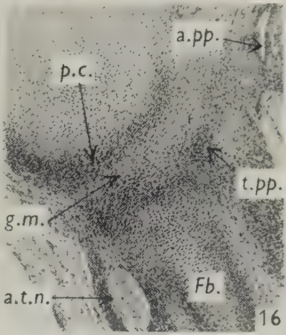


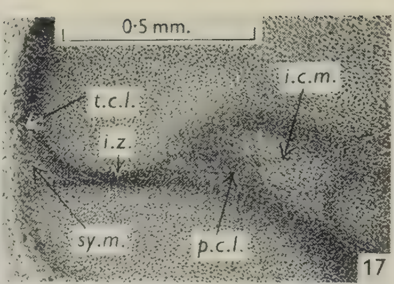
WHEELER HAINES—The early development of the femoro-tibial and tibio-fibular joints

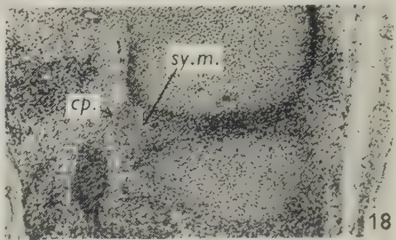


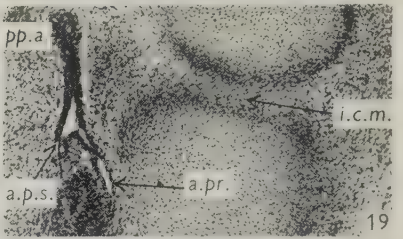


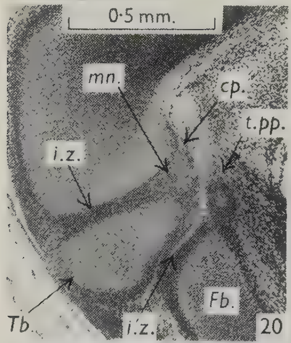


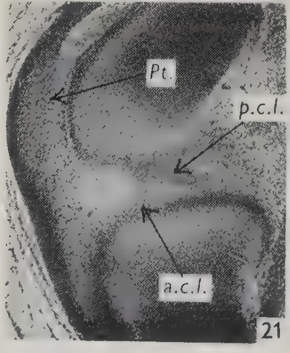


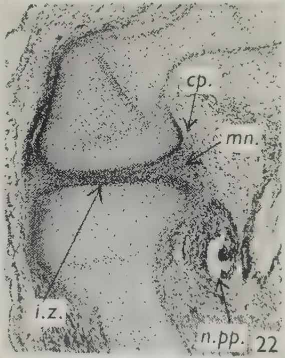


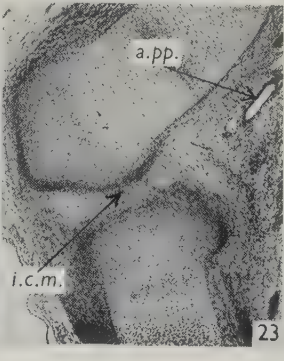


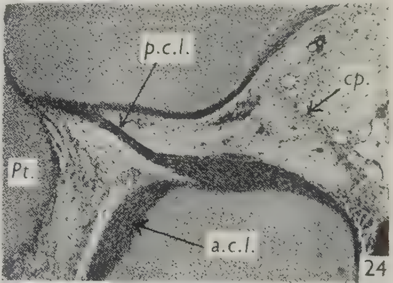


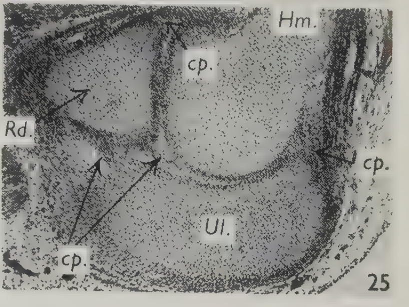












WHEELER HAINES—The early development of the femoro-tibial and tibio-fibular joints

EXPLANATION OF PLATES

PLATE 1

The scale on fig. 1 applies to all the figures on this plate.

Fig. 1. Hamilton's 12 mm. embryo 'Doyle', 44.3.4. The tibia and fibula show early chondrification, and are separated by loose mesenchyme in which the axial artery passes distally. Cf. Text-fig. 2.

Fig. 2. Davies's 13 mm. embryo 'No. 6', 32.1.3. Dense tissue occupies the femoro-fibular interval. Cf. Text-fig. 2.

Fig. 3. Hamilton's 13 mm. embryo 'Hall', 18.3.2. The arteria communicans superior passes medially to supply the adductor muscle mass. Cf. Text-fig. 3.

Fig. 4. Boyd's 13 mm. embryo 'H 23', 42.4. A dense interzone separates the medial condoyle of the femur from the tibia. Cf. Text-fig. 4.

Fig. 5. Middlesex 16 mm. embryo 'Series 1', 41.3.4. A capsule passes between the femur and tibia. Cf. Text-fig. 5.

Fig. 6. Hamilton's 16 mm. embryo 'Futcher', 18.3.5. Transverse. The intercondylar vascular mesenchyme is bounded posteriorly by a capsule.

Fig. 7. Davies's 16 mm. embryo 'No. 1', 8.2.5. Medial condylar region with medial interzone. Cf. Text-fig. 6.

Fig. 8. As fig. 7. 8.1.3. Intercondylar region.

Fig. 9. As fig. 7. 7.3.5. Lateral condylar region with lateral interzone.

Fig. 10. As fig. 7. 7.1.3. Fibular region.

Fig. 11. Hamilton's 18 mm. embryo 'Douglas', 140.4. Coronal, cutting capsule. Cf. Text-fig. 7.

Fig. 12. As fig. 11, 142.1. Femoro-fibular region with fibular collateral ligament and tendon of popliteus. Capsule joining head of tibia.

PLATE 2

The scale on fig. 17 applies to figs. 13–19 and 25, and that on fig. 20 to figs. 20–24.

Fig. 13. Davies's 20 mm. embryo 'No. 4', 18.2.2. Tibial condylar region with capsule and interzone. Cf. Text-fig. 8.

Fig. 14. As fig. 13, 17.1.3. Intercondylar region with capsule and indication of posterior cruciate ligament.

Fig. 15. As fig. 13, 16.2.3. Lateral condylar region with capsule, tibio-fibular interval with loose mesenchyme.

Fig. 16. As fig. 13, 21.2.4. Femoro-fibular interval, from the left leg.

Fig. 17. Middlesex 21 mm. embryo 'Series 8', 80.2.2. Coronal section cutting the medial collateral ligament, posterior cruciate ligament and medial condylar interzone.

Fig. 18. Hamilton's 22 mm. embryo, 77A.2.2. Lateral condylar region with capsule. Cf. Text-fig. 9.

Fig. 19. As fig. 18, 76A.2.3. Intercondylar region. Capsule deficient opposite the bifurcation of the axial artery.

Fig. 20. Boyd's 26 mm. embryo 'H 11', 77.1.5. Lateral condylar region with capsule and tibio-fibular interzone. Cf. Text-fig. 10.

Fig. 21. As fig. 20, 73.2.5. Intercondylar region. Capsule deficient opposite posterior cruciate ligament.

Fig. 22. Davies's 25 mm. embryo 'No. 5', 13.1.1. Medial condylar region. Capsule present.

Fig. 23. As fig. 22, 12.2.5. Intercondylar region. Capsule absent opposite anterior cruciate ligament.

Fig. 24. West's 34 mm. embryo, 17.3. A faintly marked capsule has reappeared in the intercondylar region.

Fig. 25. Middlesex 21 mm. embryo 'Series 8', 91.2.2. Elbow showing separate capsules for humero-radial and humero-ulnar joints.

KEY TO LETTERING

a.c.l.	anterior cruciate ligament
a.c.s.	arteria communicans superior
ad.m.	adductor muscles
a.p.p.s.	arteria peronea posterior superficialis
a.pr.	arteria poplitea profunda
a.p.s.	arteria poplitea superficialis
a.t.a.	anterior tibial artery
a.t.n.	anterior tibial nerve
a.t.p.s.	arteria tibialis posterior superficialis
ax.a.	axial artery
bic.	biceps
b.v.	blood vessel
c.l.i.	chondrogenic layer of interzone
cp.	capsule
ctg.	cartilage
e.c.p.	extra-capsular perichondrium
Fb.	fibula
fm.a.	femoral artery
f.c.l.	fibular collateral ligament
Fm.	femur
gas.	gastrocnemius
g.m.	general mesenchyme
hd.T.	head of tibia
Hm.	humerus
i.c.m.	inter-condylar mesenchyme
i.c.p.	intra-capsular perichondrium
i.z.	interzone
lig.	ligament
l.i.z.	lateral interzone
l.p.n.	lateral popliteal nerve
m.i.z.	medial interzone
m.l.i.	middle layer of interzone
mn.	meniscus
p.c.	perichondrium
p.c.l.	posterior cruciate ligament
pop.	popliteus
Pt.	patella
p.t.a.	perforating tarsal artery
p.t.n.	posterior tibial nerve
pp.a.	popliteal artery
pp.n.	popliteal nerve
Rd.	radius
sk.b.	skeletal blastema
sol.	soleus
sy.cv.	synovial cavity
sy.fd.	synovial fold
sy.m.	synovial mesenchyme
Tb.	tibia
t.c.l.	tibial collateral ligament
t.pp.	tendon of popliteus
t.q.	tendon of quadriceps
Ul.	ulna

THE FORM AND MOBILITY OF THE FIBULA IN METATHERIAN MAMMALS

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Department of Anatomy, St Thomas's Hospital Medical School

INTRODUCTION

In a previous communication (1953) the mobility of the fibula in the eutherian mammals was discussed in relation to the variations in gait shown by arboreal, fossorial, saltatorial and cursorial forms. The tibio-fibular articulations in each of these four groups showed well-defined characteristics that could be correlated with their particular functional requirements. The present investigation was undertaken to ascertain whether the principle of the adaptation of structure to function could also be demonstrated in the hind-limbs of the Marsupialia, which include many of the important adaptive types found among the eutherian mammals.

MATERIAL AND METHODS

The following species have been studied by dissection as well as in the dry state:

Common opossum, *Trichosurus vulpecula*
Spotted cuscus, *Phalanger maculatus*
Short-headed flying phalanger, *Petaurus breviceps*
Lemur-like opossum, *Pseudochirus lemuroides*
Ursine wombat, *Phascolomys ursinus*
Red-necked wallaby, *Wallabia rufogriseus*
Fawn nail-tailed wallaby, *Onychogalea unguifera*
Tree-kangaroo, *Dendrolagus dorianus*
Common rat-kangaroo, *Potorous tridactylus*
American opossum, *Didelphys marsupialis*
Tasmanian wolf, *Thylcainus cynocephalus*
Tiger-cat, *Dasyurus maculatus*
Tasmanian devil, *Sarcophilus harrisii*
Crest-tailed pouched mouse, *Dasycercus cristicauda*
Fat-tailed pouched mouse, *Sminthopsi crassicaudata*
Banded ant-eater, *Myrmecobius fasciatus*
Pouched mole, *Notoryctes typhlops*
Jerboa pouched mouse, *Antechinomys spenceri*
Short-nosed bandicoot, *Isoodon obesula*

In addition a further eleven species have been examined either as dried ligamentous preparations or as disarticulated skeletons:

Water opossum, *Chironectes minimus*
Murine opossum, *Marmosa murina*
Brush-tailed pouched mouse, *Phascogale penicillata*
Yellow-footed pouched mouse, *Phascogale flavipes*
Rabbit bandicoot, *Thalacomys lagotis*
Red kangaroo, *Macropus rufus*
Black-tailed wallaby, *Macropus ualabatus*
Rock wallaby, *Petrogale inornata*
Ursine tree-kangaroo, *Dendrolagus ursinus*
Koala, *Phascolarctos cinereus*
Hairy-nosed wombat, *Phascolomys latifrons*

Since in eutherian mammals one of the distinctions between the functional types was based upon the gross anatomy of the tibio-fibular articulations, these joints were carefully measured in the wet specimens. A further distinguishing feature noted in eutherian mammals was the relative proportions of the two limb bones, the fibula being large relative to the tibia in the burrowing and swimming forms, thin and flexible in the saltatorial forms, and intermediate in size in those primates and carnivores adapted for progression over uneven surfaces in the trees or on the ground. The dried bones of the marsupial specimens were therefore weighed separately where possible, and the findings used as indices of the relative bulk of the two bones. This quantitative method was found to be the simplest means available of expressing the relative sizes of the tibia and fibula.

FINDINGS

(1) *The superior and inferior tibio-fibular joints*

The problem of fibular mobility in the Metatheria clearly differs from that in the Eutheria, since in the former there is generally some degree of femoro-fibular contact, which is absent in the latter. This contact entails a relative displacement of the fibula and tibia during axial rotation of the distal segment of the limb, somewhat akin to the movements of pronation-supination in the human forearm. Among the eutherian mammals, on the other hand, the movement of the fibula relative to the tibia is secondary to flexion and extension of the talus within the ankle mortice. As Haines (1942) has stated, the upper and lower articulations of the fibula in Metatheria are intermediate between the eutherian and the primitive reptilian patterns. The fibula commonly articulates with the femur via a cartilaginous disc which is partly or wholly discontinuous with the lateral meniscus; a similar condition is usually present in reptiles. The tibia and fibula articulate at both upper and lower extremities, however, a condition not found in reptiles. At the upper end a synovial joint continuous with that of the knee is usually present, while at the lower end also a synovial joint exists in some species. The metatherian type of tibio-fibular relationship appears to provide a means whereby both reptilian and eutherian types of fibular movement are combined.

Although many marsupials show the general limb pattern indicated, a number of modifications exist in various species. The general trend is towards a withdrawal of the fibula from the femur, linked with a progressive increase in the functional significance of both articulations between the tibia and fibula. The characteristics of the femoro-fibular and tibio-fibular relationships provide a basis for classifying the hind-limbs of the Metatheria into three main groups.

In group 1 (Fig. 1), the fibula articulates with the tibia by means of a synovial joint, continuous with the knee cavity, the plane of which is nearly vertical in most of the species examined. The fibula articulates with the femur indirectly via a thick cartilaginous disc which is partly detached from the lateral meniscus, and which is in contact with an articular facet on the supero-medial aspect of the fibula. In some species—for example, the short-headed flying phalanger (*Petaurus breviceps*) and the Tasmanian devil (*Sarcophilus harrisii*)—this femoro-fibular disc has a distal extension, semilunar in form, which intervenes between the tibia and fibula, acting as a meniscus within the superior tibio-fibular joint. The tibia and fibula make only limited contact at their lower ends, there being usually a small synovial joint but no syndesmosis. There is no true mortice at the ankle, the talus in these forms tending to be flattened or gently rounded. As in all marsupials the fibula also articulates with a facet on the calcaneum. The fibula is stout relative to the tibia, ranging from one-third to one-half of its weight. This group includes all the marsupials in this series except the pouched mole (*Notoryctes typhlops*) and the saltatorial forms.

In group 2 (Fig. 2), the upper end of the fibula is notched, articulating with the side as well as the undersurface of the tibial condyle, which projects laterally as a broad ledge. A femoro-fibular disc, similar to that found in group 1, is usually connected by a fibrous band to the anterior horn of the lateral meniscus, and articulates with a small facet on the tip of the fibula. The syndesmosis inferiorly between the two bones extends from one-third to two-thirds of the total length of the tibia. The ankle joint is in the form of a mortice with occasionally a very small synovial extension upwards between the two bones. The fibula is extremely flexible and slender, forming about one-eighth of the weight of the tibia; it is flattened in cross-section opposite the syndesmosis and slightly concave to fit closely against the rounded shaft of the tibia. Group 2 includes the Macropodidae and one member of the Dasyuridae, the jerboa pouched mouse (*Antechinomys spenceri*).

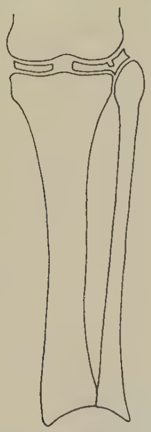

Fig. 1.

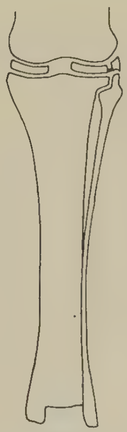

Fig. 2.

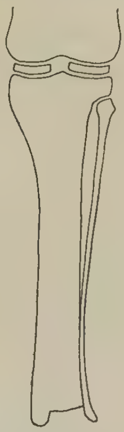

Fig. 3.

Fig. 1. The fibular articulations present in most of the marsupial genera.

Fig. 2. The fibular articulations in the Macropodidae.

Fig. 3. The fibular articulations in the Peramelidae.

In the third group (Fig. 3), the slender fibula does not articulate with the femur and the femoro-fibular disc is absent. The superior tibio-fibular joint is confined to the under-surface of the over-hanging tibial condyle. The two bones are united inferiorly as in group 2 by a syndesmosis that ranges in extent from one-quarter to one-half of the total length of the tibia. The ankle joint is in the form of a mortice; a synovial joint between the lower ends of the tibia and fibula is rarely present and was noted in only one species, the short-nosed bandicoot (*Isoodon obesula*). This group includes the Peramelidae and one member of the Didelphyidae, the murine opossum (*Marmosa murina*).

Notoryctes, the only marsupial fully adapted for a fossorial life, is not included in this classification. In this highly specialized animal the upper end of the fibula articulates, without an intervening femoro-fibular disc, with a facet on the lateral aspect of the femoral condyles as well as with the tibia (Fig. 4). Inferiorly, there is a large synovial recess extending upwards from the ankle joint between the two bones, involving one-tenth of the shaft of the tibia. The fibula articulates with the lateral half of the upper surface of the talus, which fits securely between the malleolli in a deep mortice; it is unusually stout, forming one-half of the weight of the tibia. In many respects the knee joint of *Notoryctes* resembles that of the aquatic reptile *Emys blandingii* (Haines, 1942).

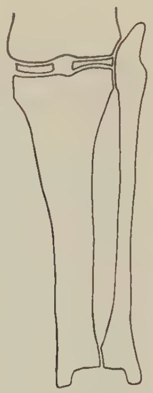

Fig. 4.

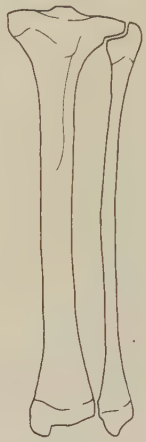

Fig. 5.

Fig. 4. The fibular articulations of the pouched mole. The fibula and femur articulate without the intervention of a cartilaginous disc.

Fig. 5. The tibia and fibula of *Dendrolagus dorianus*.

The tree-kangaroo, *Dendrolagus*, is an interesting intermediate form (Fig. 5). Its upper articulations resemble those of the other Macropodidae described in group 2, while the lower articulations and certain features of the shaft are more typical of the species in group 1. The inferior syndesmosis is relatively short (comprising less than one-tenth of the length of the tibia in *D. dorianus* and one-third in *D. ursinus*), and the fibula is stout (one-quarter of the weight of the tibia). In addition, the bone appears rounded throughout in cross-section, as in the majority of the marsupials other than the Macropodidae.

(2) *Movements of the fibula*

In the marsupials of group 1, rotation of the leg and foot upon the knee occurs mainly by differential movement of the tibia and fibula, the lower ends of the two long bones moving sagittally in opposite directions, while the upper ends undergo relatively little displacement on account of their ligamentous attachments to the femur. At the same time each bone rotates slightly about its own long axis. These movements resemble those occurring in many reptiles. Fibular mobility in this group is thus subsidiary to rotation at the knee. In contrast, fibular mobility in group 3 marsupials is clearly unrelated to rotation at the knee, since the fibula in these species is fully withdrawn from the femur. The presence of a synovial articulation between the upper ends of the bones indicates, however, that at some phase of locomotion a certain amount of movement must occur at this site. The marsupials included within group 2 show the features of both groups. Although the fibula articulates indirectly with the femur in these species, the joint between the fibula and tibia clearly constitutes the main articulation, and, as might be expected, there is only a limited amount of differential movement between the tibia and fibula during axial rotation of the distal part of the limb.

Bony fusion of the lower ends of the tibia and fibula is rarely found in the Metatheria. In the present series it was noted only in the rabbit bandicoot (*Thalacomys lagotis*) and in one of two specimens of the murine opossum (*Marmosa murina*). A similar condition has been reported in only two further genera—*Hypsiprymnodon* (Owen, 1866) and *Choeropus* (Parsons, 1903). No instance of fusion of the upper ends of the bones has been observed in this series.

DISCUSSION

In the reptiles, an alteration in the posture of the foot is brought about by relative displacement of the tibia and fibula, together with a certain amount of independent axial rotation of the two bones (Haines, 1942). A similar independent mobility of the two limb bones has evidently been retained in the majority of the metatherian genera, which have been able to utilize the primitive arrangement, with few modifications, irrespective of their mode of progression. Thus within group 1 are found marsupials adapted for arboreal life (for example, the phalangers and the koala), for a relatively unspecialized terrestrial existence (for example, the Tasmanian wolf) and for aquatic life (for example, the water opossum). There is remarkably little morphological difference between the form and mobility of the fibula in these ecologically distinct species. It can be inferred that the tibio-fibular relationship found in this group approximates closely to the ancestral marsupial pattern.

It is only among the jumping species that major deviations from the primitive arrangement are found, and the inference is that the unmodified limb is ill-suited for saltatory progression. In marsupials of groups 2 and 3, the presence of a slender flexible fibula, an extensive inferior syndesmosis and a large synovial superior tibio-fibular joint fulfil all the criteria of a saltatory hind-limb found so commonly among the eutheria. The manner in which this peculiar arrangement may contribute towards jumping ability has been discussed elsewhere (Barnett & Napier, 1953); it was suggested that the flexibility of the fibula helped to increase the initial velocity of joint movement.

The unique tibio-fibular articulations of the tree-kangaroo (Fig. 5) are probably explicable by the fact that this form is shedding some of the specializations common to the Macropodidae in favour of others more suited to an arboreal existence. It is generally accepted that the Marsupialia were originally fitted for life in the trees, but the primitive mobility of the hind-limb was necessarily lost when the kangaroos adopted a saltatory gait. Axial rotation of the distal segment of the limb was reduced, and movement of the foot restricted to one vertical plane. Consequent upon the resumption by the tree-kangaroo of an arboreal mode of life, in which a foot that is capable of a wide range of flexion and extension and able to bear inversion and eversion strains is a prerequisite, a new method of accommodating the foot to uneven surfaces has been evolved, resembling that found in eutherian arboreal forms. The authors have previously shown that in the Eutheria such a foot necessitates the presence of a mobile fibula, which can undergo axial rotation as the foot is dorsiflexed. In a wet specimen of *Dendrolagus* the existence of an exactly similar axial rotation was confirmed by direct experiment: as the foot was passively dorsiflexed a lateral rotation of the fibula took place, amounting to 2–3 degrees. The short syndesmosis and the rounded form of the fibular shaft permitted this movement to occur. As in most of the primates, the lateral side of the talus of *Dendrolagus* is not perpendicular to the axis of rotation at the ankle, and the plane of this surface must therefore change as the foot is dorsiflexed. It follows that the fibula must rotate to maintain stable contact between the two bones.

Jones (1929) has pointed out that *Dendrolagus* has been unable to retrace the steps by which a primitive arboreal marsupial gave rise to the saltatory Macropodidae. The modifications that have occurred in the hind-limb exemplify this rule. Structural changes fitting the limb for a saltatory mode of progression involved a reduction of the primitive freedom of movement of the foot. Since an entirely new method of adapting the limbs to the branches of trees was evolved when the genus resumed an arboreal life, it must be assumed that the changes involved could not be reversed.

The morphological resemblances between the fibulae of mammals with similar habits of progression provide examples of convergent evolution. There is a striking similarity between the hind-limb of the common rabbit (*Oryctolagus cuniculus*) and that of the rabbit bandicoot (*Thalacomys lagotis*); in both species, the lower end of the thin, flexible fibula is incorporated into the tibia. The relative proportions of tibia and fibula in *Dendrolagus* are similar to those of the arboreal primates. Again, the massive nature of the fibula in the marsupial mole closely resembles that of the eutherian moles, although the characteristic fusion of the tibia and fibula in the latter species is not present in the metatherian counterpart. A further example of convergent evolution within the order of Marsupialia itself is provided by the development, apparently independently (Bensley, 1903), of similar specialized modifications in the fibulae of the jerboa pouched mouse (*Antechinomys spenceri*), among the Dasyuridae, in the murine opossum (*Marmosa murina*), among the Didelphyidae, and in most members of the Macropodidae and Peramelidae. The form and mobility of the fibula in these saltatory marsupials is analogous to that occurring among the Eutheria in such diverse species as the tarsier, jerboa, hare and jumping-shrew.

In general, the form of the fibula and its articulations are remarkably uniform throughout the present series. The rarity of bony fusion between the tibia and fibula indicates that in this respect the Metatheria have retained the typical tetrapod limb with two independent bones. This is in contrast to the Eutheria where highly specialized modes of progression have led to radical departures from the original reptilian arrangement. The retention of an unspecialized hind-limb in so many of the metatherian species could indicate either an inadequate period of time for widespread adaptive radiation to have occurred or, on the other hand, an environment which did not demand extremes of locomotory specialization. The latter is the more likely explanation for, as Simpson (1950) has stated, 'the opportunities offered by Australia were obviously not as extensive or varied as those offered by all the other continents put together'.

SUMMARY

1. The hind-limbs of thirty species of metatherian mammals were examined, special attention being paid to the gross anatomy of the femoro-fibular and tibio-fibular joints and to the relative proportions of the two leg bones.

2. The basic pattern of the tibio-fibular relationship in the marsupials represents a condition intermediate between that of the reptiles and the eutherian mammals. The fibula usually articulates with the femur through a cartilaginous disc as in the reptiles, but in addition makes contact with the tibia at both extremities of the bone as in the Eutheria.

3. Divergencies from this pattern provide a basis for classifying the hind-limbs of the Metatheria into three main groups. These are discussed, and the movements of the tibia and fibula in each are considered and compared with the condition found among the reptiles. *Notoryctes*, being unique in several respects, is described separately.

4. The majority of species examined showed a remarkable uniformity of tibio-fibular relationship; only among the jumping forms were major deviations found. The articulations of *Dendrolagus* are discussed in the light of Jones's statement that this animal has been unable to 'retrace its structural steps and become anatomically a primitive arboreal mammal'.

5. Morphological resemblances between those representatives of Metatheria and Eutheria that pursue the same mode of life are indicated.

6. It is suggested in conclusion that the retention by the majority of species of a primitive marsupial tibio-fibular pattern is the outcome of the limited opportunities for functional adaptation offered by the Australian continent.

We are greatly indebted to Prof. F. Wood Jones for granting us access to his collection of marsupial material, and for his advice in the preparation of this paper. Our thanks are due also to Dr F. C. Fraser and Miss J. E. King of the Osteological section of the Zoology Department of the British Museum for the material help they have given us, and to Prof. D. V. Davies for criticism and advice. Mr A. L. Wooding is responsible for the photographic work.

REFERENCES

Barnett, C. H. & Napier, J. R. (1953). The rotatory mobility of the fibula in eutherian mammals. *J. Anat., Lond.*, **87**, 11–21.

Bensley, B. A. (1903). On the evolution of the Australian Marsupialia. *Trans. Linn. Soc. Lond.* (*Zool.*), **9**, 83–217.

Haines, R. W. (1942). The tetrapod knee joint. *J. Anat., Lond.*, **76**, 270–301.

Jones, F. W. (1929). *Man's Place among the Mammals.* London: Edward Arnold and Co.

Owen, R. (1866). *Comparative Anatomy and Physiology of Vertebrates*, **2**. London: Longmans Green and Co.

Parsons, F. G. (1903). On the anatomy of the pig-footed bandicoot (*Choeropus castanotis*). *J. Linn. Soc.* (*Zool.*), **29**, 64–80.

Simpson, G. G. (1950). *The Meaning of Evolution.* London: Oxford University Press.

REVIEWS

Regeneration and Wound-Healing. By A. E. NEEDHAM. (Pp. viii + 152; 4 illustrations; $6\frac{1}{2} \times 4\frac{1}{4}$ in.; 8*s.* 6*d.*) London: Methuen. 1952.

Dr Needham has earned the gratitude of his fellow biologists with his admirable review on the subject of Regeneration and Wound Healing in animals. The whole literature of this field is large and widely scattered; Dr Needham's bibliography extends to over 550 references. The scope of this work extends from wound healing in man to morphallaxis, or remodelling after injury in Protozoa and Hydroids; each topic is treated, wherever possible, both at the microscopical and biochemical levels. In a final chapter, the whole question is discussed from the evolutionary angle.

Although, in general, the power of regeneration decreases with ascent of the evolutionary scale, yet there is considerable variation in this respect, as for instance among Protozoa and among related groups of flatworms. Possibly an inquiry into the reasons for these discrepancies might lead to further understanding of the whole subject. At any rate, Dr Needham has prepared the way for the renewed study of many aspects of phenomena of regeneration.

Two small points of criticism are that Fischer's work on the regeneration of tissue cultures might well have been discussed, and an index of authors as well as of subjects would have been helpful.

ARTHUR HUGHES

Cowdry's Problems of Ageing: Biological and Medical Aspects, 3rd edition, edited by A. I. LANSING. (Pp. xxiii + 1061; $15.00.) Baltimore: The Williams and Wilkins Company.

Under new editorship this 3rd edition of *Cowdry's Problems of Ageing* is substantially a new work. Of the 47 contributors only 14 were represented in the 2nd edition which appeared in 1942, and the first four chapters of that edition, in which the features of ageing in plants and in the principal zoological groups were systematically described, have been omitted. The editor states that his principal objective has been to 'render a report on what has transpired in gerontology since 1942', and it is from that point of view that the book must be considered.

Dr Lancing has re-arranged the contents in three major sections, the first of which deals with biological and cellular problems, the second with clinical and organic problems and constitutes about 70 % of the book, and the third with the social and economic problems of ageing. Apart from a short chapter on general physiology by Dr Lansing, the first section is made up of chapters all of which had appeared before, but brought up to date and in some cases considerably increased in length. This section contains Prof. Cowdry's own contributions on the ageing of tissue fluids and individual cells, and an important chapter on the chemical aspects of ageing and the effects of diet by Prof. McCay. The second section contains 29 articles on different systems and organs, on certain clinical conditions such as arteriosclerosis and cancer, and on such general topics as 'Degeneration and Regeneration' and 'Surgical Problems in the Aged'. The last and shortest section on social and economic problems is sufficiently described by its title.

It is perhaps inevitable that a work of this kind, covering so wide a field and with so many different contributors, should lack unity. One gets an impression of an enormous collection of miscellaneous facts, many of them of great interest and importance, but not yet assimilated to any body of systematic knowledge. Important though they are, it is perhaps difficult to bring to a study of the problems of ageing the enthusiasm which is characteristic of embryologists, and this may account for the 'spirit of pessimism' which the editor finds in many of the chapters, and also for his statement that 'research in the biology of ageing is almost at a standstill'. In spite of this, however, the book is valuable in bringing together, with full bibliographies, many facts which are at present known about the ageing processes in animals, although the progress reported since 1942 is a little disappointing. It is certainly to be hoped that the book will fulfill the wish expressed by the editor, that it will stimulate new research in a subject which is becoming of increasing concern to the ageing populations of Europe and America.

F. GOLDBY

The Mitotic Cycle. The Cytoplasm and Nucleus during Interphase and Mitosis. By ARTHUR HUGHES, M.A., Ph.D. (Pp. viii + 232; 69 figures; 10 × 6¼ in.; 35*s*. net.) London: Butterworths Scientific Publications. 1952.

Dr Hughes prefaces his text with, amongst other quotations, one by D'Arcy Thompson: 'We have learned many things about cell division, but we do not know much in the end.' In view of such a statement a special admirable courage is needed to embark on the unenviable task of gathering together and of attempting to evaluate the numerous new data, morphological and physiological, pertaining to the process of cell division, which have accumulated in more recent years. The task may have been made somewhat easier for the author through the impetus given by his own brilliant experimentation in this field of cytology, much of which is incorporated in the main sections of this book. Everybody who has seen some of Dr Hughes's beautiful and instructive phase-contrast films of living, resting and dividing cells will be pleased to see—besides many other illustrations—so many of the frames here reproduced, and will be able to enliven these pictures by recollecting the cells in action. Each plate showing such frames is suitably supplemented by explanatory outline drawings, of the essential structures, facing the micro-photographs—a most helpful device.

Apart from the review of the more recent literature, historical flash-backs are given which link the modern observations with the first classical achievements in this field. In these historical parts a more consistent mentioning of dates would have been desirable.

Throughout the book, the physiological side of the subject has received special emphasis. In keeping with this the book opens with a chapter on the nucleic acids. In chapter 2, 'The Interphase cell and the Cytoplasm', work concerning the Golgi body, mitochondria, vacuoles (though the two microphotographs supposed to show pinocytosis are not convincing) and the ultra-structure of protoplasm is reviewed. Then follows a chapter on the Interphase Nucleus, in which, amongst other things, the discussion of nucleic acids is carried further and extended to the nuclear proteins. Valuable criticism is brought to bear on the methods of quantitative estimations of the former, and also on the validity of the techniques of isolation, from resting cells, of chromosomes, but not on the controversial problem of nuclear phosphatases.

The bulk of the book (chapters 4 and 5) is devoted to cells in division and the experimental analysis of the process by means of promoters and inhibitors. These chapters alone list nearly 700 references. Every conceivable aspect is touched upon and a mass of information presented. That the reader is sometimes in fear of losing the thread and being left guessing, is due just to this amazing maze of data and observations, obtained by a great variety of techniques from vastly different types of cells, a proper correlation and synthesis of which must—as the author himself states—still be left to the future. However, the expert in the field will not only find this comprehensive review invaluable, but also in it much food for thought, and, almost on every page, problems are indicated which await the enthusiastic experimentator.

Dr Hughes had the support of two outstanding experimental scientists. Prof. Swann has provided a lucid and critical account of the structure of the spindle and its dynamics, i.e. the forces possibly at work which bring about the anaphase movements of the chromosomes.

Dr Waymouth had the difficult task of dealing with the data concerning the nature of the mitotic stimulus, a field which, in spite of the enormous efforts which have been made for its elucidation, is still almost as obscure as the mechanism of the visible process of mitosis. By a judicious selection from the literature in which naturally tissue culture work figures largely, the many facets of the problem are shown up by searching lights. The confusion and contradictions which exist are revealed perhaps most clearly in the section on leucocytic secretions.

Minor points of criticism: it is strange to see the references listed not in alphabetical order, but according to their occurrence in the text, which occasionally involves duplication. A few gross typographical errors have escaped notice.

Fascinating and stimulating as this account is of the past work on the mitotic cycle, one is continually made aware that any future progress in this field—more than in many others—is bound to be very slow, full of pitfalls and very arduous.

F. JACOBY

Die Entwicklung des braunen Grasfrosches, Rana fusca *Roesel*. By F. Kopsch, 1952. viii + 70 Seiten, 32 Abb., 2 Tafeln. Preis: Halbleinen, D.M. 48.

This monograph on the development of *Rana fusca* is in the great tradition of the Keibel *Normentafeln* and will doubtless be as useful to embryologists as the sixteen volumes of that great mine of carefully recorded facts on vertebrate development. In his introduction Prof. Kopsch explains how the present volume was planned over fifty years ago. Much of the material and many of the drawings go back to the period of that early intent. For reasons explained, the appearance of the volume has been delayed until now. The delay, however, has not been to the disadvantage of the presentation. Those who will have to use this book in their work will feel all the more confidence in the extra thought and the ripeness of experience that have gone into it.

A detailed review of such a monograph is not possible. The presentation is much on the lines of the Keibel volumes. The inclusion of a synopsis of the European members of the Anura, and in particular a detailed comparison of *R. fusca* and *R. arvalis*, will make the present volume welcome to herpetologists. For embryologists it will be invaluable, both as a meticulous account of the development of a particular amphibian type, and as a guide for comparisons with the ontogeny of related types. The plates, the drawings of which are due to Müller, Krause and the author, are most attractive.

Prof. Kopsch is to be congratulated on a volume that adds to the lustre of the great German tradition of detailed morphological embryology. Without the base-line provided by such work, experimental embryology could hardly flourish.

J. D. BOYD

Fibre Systems of the Brain and Spinal Cord. By M. K. Wright, M.Sc., M.B., B.Ch., (Pp. 103; 15 text-figures; 21 plates; 41*s*.) Johannesburg: Witwatersrand University Press. 1952.

This is a students' text-book, designed to illustrate the essential details of the tracts and fibre connexions in the central nervous system which are of importance for the medical student. The microphotographs of Weigert sections are excellently reproduced and, together with a number of singularly effective diagrams, make clear the basic organization of the brain and spinal cord. The descriptive accounts are well-balanced, clear and concise, but the author has taken care not to sacrifice accuracy for the sake of brevity and has made due reference to the results of the most recent neurological investigations bearing on the subject.

W. E. LE GROS CLARK

Lillie's Development of the Chick—an Introduction to Embryology. 3rd Edition, revised by Howard L. Hamilton. (Pp. 574; 283 figs.; 14 plates; $8.50.) New York: H. Holt & Co. 1952.

The writing of the present edition was begun in 1945 at the request of Dr Frank R. Lillie himself with Dr B. H. Willier acting as advisory editor. It was Dr Lillie's hope that he might live to see the new edition in print but this was not to be. The general outline of previous editions has been preserved. Part 1, which consists of six chapters, is devoted to an account of the early embryology up to and including the 3rd day. The account of the development of the embryo is given on a general basis and in addition a detailed account is given of specially selected stages.

Part 2 of the book consists of nine chapters and is an account of the development of the embryo from the 4th day to hatching; the various systems and external form are described as separate entities. A few chapters, such as the one dealing with the external form of the embryo and the embryonic membranes, and the one describing the body cavities, mesenteries and septum transversum, have remained relatively unchanged. Chapter 4, 'From laying to the formation of the first somite', chapter 8; 'The nervous system', and chapter 13, 'The urogenital system', are more or less completely rewritten. A new chapter, the fifteenth, describing the development of the integument, has been added. The other chapters have been extensively revised.

The new accounts are based on recent literature, but the author has tried to follow Dr Lillie's example of going to the chick itself to check questionable points. To this end some original work is included in the text, but it is to be regretted that the author has not indicated more clearly which parts of the text result from this original work. The only clear indications consist of an opinion on the processes concerned with the formation of endoderm (p. 101) and two footnotes, one dealing with the coelomic cavity (p. 149) and one with the tail bud (p. 176). A further footnote refers to a communication from Rawles on the patency of the ductus arteriosus in the newly-hatched chick (p. 462).

This book is very well written and its format is attractive. The book reaches a happy compromise which makes it a most readable introduction to embryology while yet remaining an invaluable reference work for the research worker.

There is little to criticize in this work which has evidently been prepared with great care, but future editions might be improved by a rearrangement of the bibliography. The references should be listed at the end of the chapter they concern and not in an appendix of 32 pages at the end of the book. Also the magnification of drawings and photographs of early embryos should be given. Figs. 153 and 155 would be improved by being photographs rather than drawings of sagittal sections through an embryo. In fig. 222 the drawings are too small and too faint.

Apart from these minor faults the present work is a credit to the author and had Dr Lillie lived he would have been proud to have his name associated with it. It will continue to perpetuate Dr Lillie's influence on the development of embryology. W. J. HAMILTON

BOOK RECEIVED

The Editor acknowledges receipt of the first number of the *Journal of the Anatomical Society of India*. On behalf of the Editorial Committee of the *Journal of Anatomy* he congratulates the Anatomical Society of India on the production of this first number and wishes them success with their new venture. The editor of the new Journal is Dr Hiren Chatterjee, Department of Anatomy, Nilratan Sarkar Medical College, Calcutta, 14.

THE ARTERIAL SUPPLY TO THE STELLATE GANGLION

By E. L. PATTERSON
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Various branches of the subclavian artery have been mentioned by other investigators as contributing, constantly or variably, to the blood supply of the inferior cervical and first thoracic sympathetic ganglia, either as separate ganglia or united as the stellate ganglion. There is, however, little information as to the manner in which the ganglionic arteries arise and the extent of their contribution. Moreover, those who have made observations on the blood supply of the sympathetic trunk in this region have, with the exception of de Souza (1932), failed to identify consistently the ganglion which is known to-day as the inferior cervical ganglion. It has been confused on frequent occasions with a ganglion intermédiaire or with a low middle cervical ganglion. This confusion, to which attention was drawn in a previous paper (Patterson, 1950), makes the interpretation of many observations on the blood supply to this ganglion difficult. It was therefore decided to re-examine the matter and to identify the inferior cervical ganglion, in those cases in which it remains separate from the first thoracic, both by its position posterior to the vertebral artery and by its rami communicantes to the 7th and 8th cervical nerves.

MATERIAL AND METHODS

Still-born infants were used. A 10% suspension of silver iodide (with gelatin), warmed to 45° C., was injected into the ascending aorta, the pulmonary trunk having been previously ligatured. A pressure not exceeding 100 mm. of mercury was used and the injection was stopped when the suspension appeared at the edges of the mid-sternal incision. Approximately 30–40 ml. were normally required, the quantity depending upon the size of the foetus.

The ganglia and their nutrient arteries were dissected bilaterally since there is always some asymmetry both in the anatomy of the ganglia and in the arrangement of the ganglionic vessels. The latter, together with their visible ramifications on the ganglia, were drawn *in situ*, after which the ganglia were dissected free and mounted on waxed tissue paper for micro-radiography. The following observations are based on the examination of forty ganglia.

OBSERVATIONS

Whether the inferior cervical ganglion is, or is not, demarcated macroscopically from the first thoracic ganglion by a constriction of the sympathetic trunk does not materially affect the sources and arrangement of their nutrient arteries. Moreover, so free are the connexions between the arteries of the two ganglia that a descriptive subdivision of their blood supply would be quite artificial. It is proposed, therefore, to describe the vessels of the conjoined ganglia (stellate ganglion) rather than those of the two ganglia separately.

Like other sympathetic ganglia, the stellate ganglion possesses a rich blood supply, its nutrient vessels anastomosing freely both on the surface of and within it. These vessels are chiefly and constantly derived from the branches of the costo-cervical trunk, especially the superior intercostal artery. Eighteen of the forty ganglia dissected received an additional supply from the inferior thyroid artery or thyro-cervical trunk, and eight from the first aortic intercostal artery. In six cases the internal mammary artery made a small contribution along the ansa subclavia, and in four cases a mediastinal branch of the subclavian artery provided vessels to it. There is, therefore, considerable variation in the accessory sources of supply.

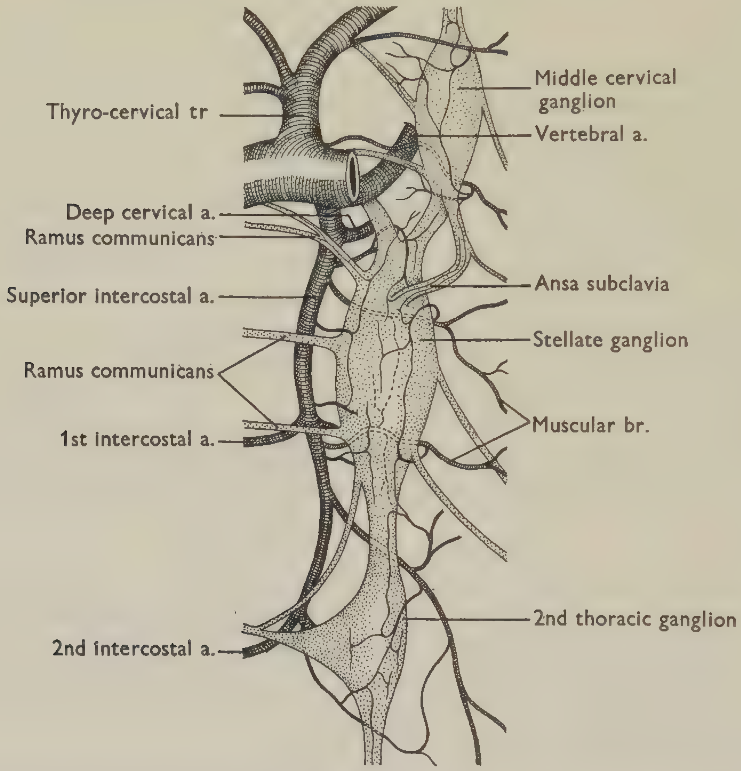

Fig. 1. Drawing from a dissection of the right stellate ganglion and adjacent arteries (new-born). The subclavian artery has been divided and withdrawn from behind the ansa subclavia. Vessels on the posterior aspect of the ganglion are indicated by interrupted lines.

Although the superior part of the ganglion is intimately related to the vertebral artery, no ganglionic vessels from the latter have been seen: in fact this artery is consistently devoid of any branches while in relationship with the sympathetic trunk.

The arrangement of the ganglionic arteries derived from these various sources will now be described.

The superior intercostal artery

This artery is a constant source of ganglionic vessels, but the precise arrangement of these is variable. It is, however, possible to group them into three sets, according to their initial relationship to the ganglion:

(*a*) *Lateral set.* One, two, or occasionally three ganglionic branchlets often arise directly from the trunk of the superior intercostal artery as it descends near the lateral margin of the ganglion (Figs. 1, 4). They pass medially and ramify on the anterior and posterior surfaces of the ganglion, more especially on the former, anastomosing with each other and with ganglionic vessels from other sources and forming with them a delicate vascular rete. When more than one of these direct lateral vessels are present, the superior vessel commonly arises above the point of origin of the first intercostal artery and the inferior below (Fig. 1), except of course in those cases in which the parent vessel fails to provide the posterior intercostal artery to the second space.

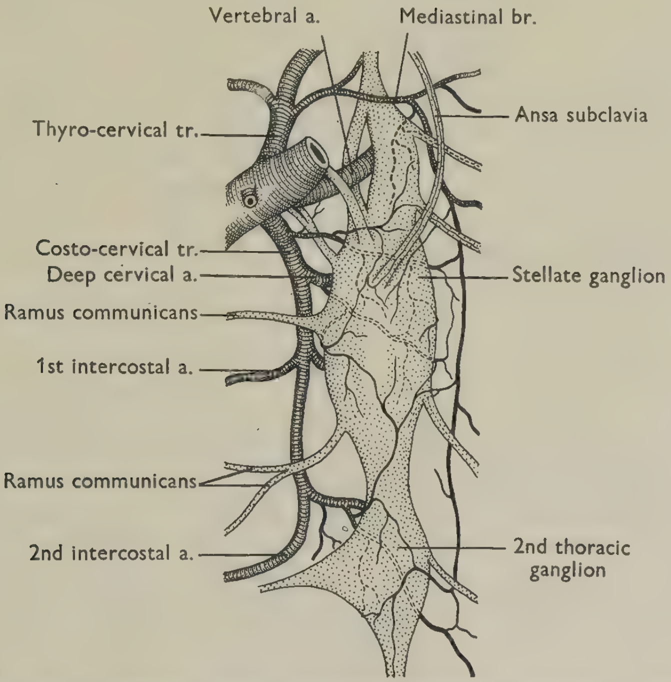

Fig. 2. Drawing from a dissection of the right stellate ganglion (new-born), showing nutrient arteries from the costo-cervical, deep cervical, superior intercostal (posterior ramus) and mediastinal branch of the thyro-cervical trunk.

Direct lateral ganglionic arteries were observed in the case of twenty-two of the forty ganglia dissected, a single vessel being present in twelve cases, two vessels in eight cases, and three in two cases.

(*b*) *Posterior set.* Posterior ganglionic arteries are constantly present but variable in number (usually 3–5). They arise on a plane posterior to that of the ganglion from the various branches of the superior intercostal artery that pass behind the ganglion.

These branches include: (1) the posterior rami (Figs. 3, 4); (2) muscular branches from the trunk of the superior intercostal artery (Fig. 1), or from its posterior rami (Fig. 2), that pass medially behind the sympathetic trunk to supply m. longus cervicis and the adjacent costo-central joints; (3) a branch, very commonly present, that arises immediately below the deep cervical artery and accompanies the latter posteriorly above the neck of the first rib (Figs. 1, 3).

Some of these posterior ganglionic vessels ramify on the dorsal aspect of the ganglion (Fig. 1), while others may curl round its lateral border to reach its ventral aspect: the latter are always to be seen in the absence of the lateral ganglionic arteries described above (Figs. 2, 3). In a similar manner others may turn round the medial border of the ganglion to reach its ventral surface, especially when a separate set of medial ganglionic arteries is lacking.

(*c*) *Medial set.* These are several small vessels that arise on the medial side of the ganglion from muscular branches supplying m. longus cervicis, and pass laterally to reach the anterior and posterior surfaces of the ganglion, upon which they ramify. In approximately a third of the dissections the parent vessels were the muscular branches from the trunk or posterior rami of the superior intercostal artery, passing behind the ganglion to reach m. longus cervicis as described above (Fig. 1). In another third of the dissections the parent vessels were from other sources, such as the inferior thyroid, subclavian, deep cervical or first aortic intercostal arteries, as described subsequently. In the remaining third, no separate set of medial ganglionic arteries were present.

The costo-cervical trunk

This artery provided branchlets to the upper part of the ganglion in approximately a third (fourteen out of forty) of the dissections. In addition, there were four cases in which the deep cervical artery arose independently from the subclavian and in which a ganglionic supply was derived from the segment of the superior intercostal artery that is normally the costo-cervical trunk. The branch concerned (Figs. 2, 4) passes medially and crosses the upper part of the ganglion either posteriorly (twelve cases) or anteriorly (six cases), giving off several branchlets to both surfaces of the ganglion. After crossing the ganglion it is distributed to m. longus cervicis and adjacent connective tissues.

The deep cervical artery

The deep cervical artery commonly supplied the upper part of the ganglion in the absence of a supply from the costo-cervical trunk (twenty ganglia); six ganglia received branches from both arteries, one of which is illustrated in Fig. 2. The ganglionic vessels, two or three in number, arose either directly from the deep cervical or from a muscular branch that crossed the ganglion, usually posteriorly, to reach m. longus cervicis (Fig. 2). They were distributed to both surfaces of the ganglion.

The thyro-cervical trunk and inferior thyroid artery

There are constant anastomotic connexions along the inferior interganglionic segment of the cervical sympathetic trunk and along the ansa subclavia between the arteries of the stellate ganglion on the one hand, and the branches of the inferior thyroid to the middle cervical ganglion (or ganglion intermédiaire) on the other.

Apart from these connexions, the inferior thyroid artery or thyro-cervical trunk played a substantial part in the blood supply of the stellate ganglion in eighteen of the forty dissections through a mediastinal branch. This vessel commonly arose near the commencement of the inferior thyroid (fourteen dissections) as in Fig. 3, but sometimes from the thyro-cervical trunk (Fig. 2). It took an oblique course medially and inferiorly, passing behind the subclavian artery, and crossed the sympathetic trunk a short distance above the stellate ganglion. It passed behind the trunk in about two-thirds of the dissections in which it was present and anterior to it in the rest. On reaching the surface of m. longus cervicis, which it supplied, it gave off a descending branch on the medial side of the stellate ganglion (Figs. 2, 3) and also branches to adjacent mediastinal structures, often including the oesophagus. The descending branch contributed several medial ganglionic arteries to both aspects of the ganglion (Figs. 2, 3) and usually anastomosed freely with branches of the superior intercostal and first aortic intercostal arteries. In these cases the ganglion was situated within an arterial ring, an arrangement reminiscent of that observed in the case of the superior cervical ganglion (Patterson, 1950).

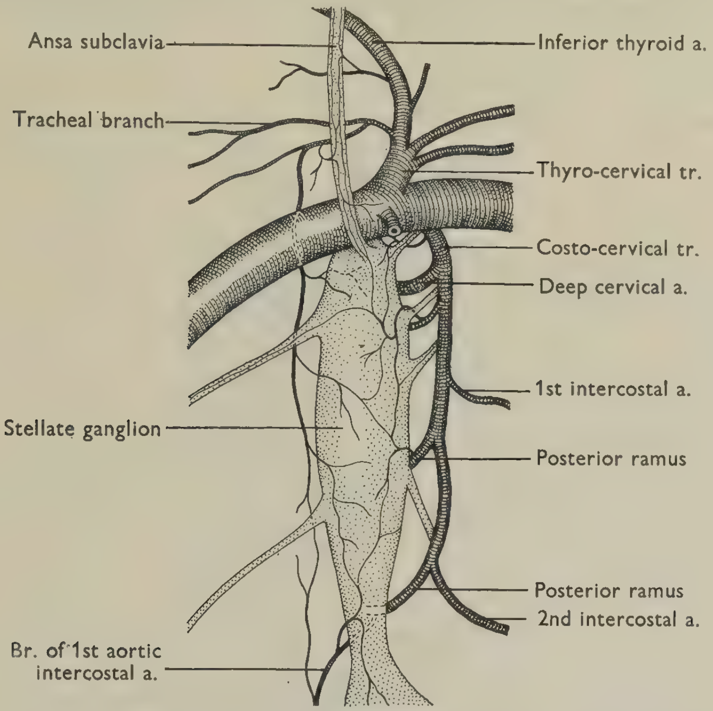

Fig. 3. Drawing from a dissection of the left stellate ganglion (new-born), showing nutrient arteries from the costo-cervical, superior intercostal, mediastinal branch of inferior thyroid and first aortic intercostal arteries.

In a further four dissections (two of which were in the same infant) a similar mediastinal branch arose directly from the *subclavian artery* near the root of the thyro-cervical trunk (Fig. 4) and supplied the ganglion from the medial side in the same way.

The first aortic intercostal artery

When the segmental artery of the second intercostal space is a member of the aortic series, it constantly provides nutrient arteries to the second thoracic ganglion and usually to the lower part of the stellate ganglion also (eight out of forty dissections). Immediately before passing behind the sympathetic trunk in these cases, the first aortic intercostal gives off a vessel that ascends on the medial side of the trunk (Fig. 4). This ascending branch anastomoses with branches of the superior intercostal artery behind the stellate ganglion and on the surface of m. longus cervicis and also, in the latter situation, with the mediastinal branch of the inferior thyroid (or thyro-cervical trunk) when this is present. It also gives off one or more medial ganglionic arteries that ramify on the anterior or posterior surface of the stellate ganglion (Fig. 4). The chief nutrient artery to the second thoracic sympathetic ganglion usually arises separately from the first aortic intercostal in these cases but has easily visible connexions with the arteries of the stellate ganglion (Fig. 4).

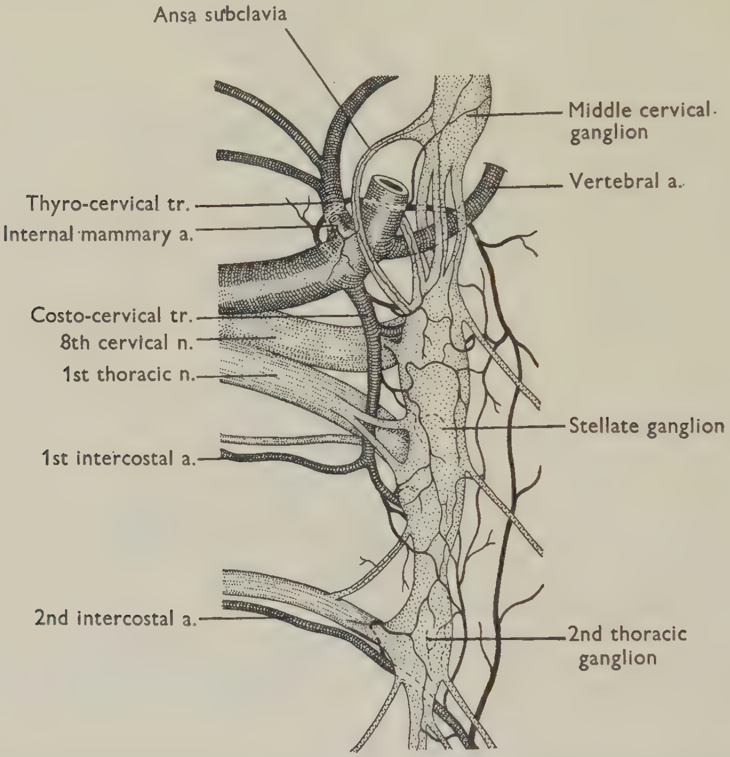

Fig. 4. Drawing from a dissection of the right stellate ganglion (new-born). The second intercostal artery belongs to the aortic series. Nutrient arteries are provided by the costo-cervical, superior intercostal, first aortic intercostal and a mediastinal branch of the subclavian artery. The arterial ring surrounding the ganglion is well illustrated.

The internal mammary artery

In six dissections this vessel had anastomotic connexions with the arteries on the ventral surface of the stellate ganglion along the ansa subclavia. In these cases a branchlet arose from the internal mammary near its origin and passed medially, being distributed chiefly to the connective tissue around the first part of the subclavian artery. From this a small vessel joined the ansa and divided dichotomously into ascending and descending channels (Fig. 4). The former anastomosed with branches of the inferior thyroid, etc., supplying the middle cervical ganglion, while the latter anastomosed with the branches of the superior intercostal, deep cervical or costo-cervical trunk on the anterior aspect of the stellate ganglion. In the remaining dissections, in which the internal mammary artery did not supply the ansa, there were direct connexions along this nerve between the arteries of the middle cervical and stellate ganglia.

DISCUSSION

Among those who have studied the vascularization of this part of the sympathetic trunk, de Souza (1932) seems to have been the first to recognize the intimate relationship between the inferior cervical and first thoracic ganglia or their fusion into a stellate ganglion, and his observations, summarized below, are readily understood. Bartholdy (1897), Delamere & Tanasesco (1906) and Anserow (1925) were unanimous in regarding the inferior thyroid artery as a constant source of supply to what they named the inferior cervical ganglion; but from their descriptions and illustrations it seems certain that the ganglion to which they referred was sometimes situated anterior to the vertebral artery or even in the region of the loop of the inferior thyroid artery and was therefore either the middle cervical ganglion or the ganglion intermédiaire.

Delamere & Tanasesco also mentioned the ascending cervical and deep cervical arteries as variable sources of supply to the 'inferior cervical ganglion', but again the identity of the ganglion is in doubt.

In regard to the arteries supplying the first thoracic ganglion, previous workers other than de Souza have made the briefest of statements. Thus Lobstein (1823) mentioned branches of the subclavian artery, and Anserow the costo-cervical trunk and superior intercostal artery, but no details were given.

De Souza's observations are more specific: the inferior cervical ganglion is generally fused with the first thoracic to form the stellate ganglion, which receives a constant supply from two sources, the inferior thyroid and superior intercostal arteries, and an inconstant supply from the vertebral artery. Of the inferior thyroid source he says that the vessels may arise directly from the trunk of the artery or from an oesophageal branch that runs medially and inferiorly, passing behind the ganglion: before crossing the ganglion this branch gives off several small vessels to it. In the present series the inferior thyroid artery (including the thyrocervical trunk) supplied the ganglion in less than half the cases, apart from the constant anastomotic vessels running in the sympathetic trunk and ansa subclavia. Moreover, direct branches from the trunk of the artery to the ganglion were not present. The occasional presence of the vessel, called by de Souza 'an oesophageal branch of the inferior thyroid', is confirmed, but the present writer disagrees with de Souza's description of it in four respects: (1) it may sometimes arise from the thyro-cervical trunk or even directly from the subclavian artery; (2) it should rather be called a mediastinal branch since it does not constantly supply the oesophagus; (3) it usually crosses the sympathetic trunk a short distance above the ganglion and is sometimes anterior to the trunk at the point of crossing; (4) it gives off its ganglionic branches to the stellate ganglion on the medial side of the latter, after having crossed the sympathetic trunk.

In regard to the superior intercostal artery, de Souza simply mentions that as it descends near the lateral border of the ganglion it provides branches that pass medially to reach the latter. These are presumably those described as the direct lateral ganglionic branches in the present re-investigation: de Souza did not apparently recognize the posterior and medial ganglionic branches of this artery described above. Nor did he report a variable supply from the costo-cervical trunk or deep cervical artery.

The present work has consistently failed to substantiate any branches of the vertebral artery supplying either the inferior cervical or stellate ganglia, which, according to de Souza, take part, though not constantly, in the blood supply of the upper part of the stellate ganglion.

De Souza recorded that the *second* thoracic sympathetic ganglion may receive branches from the superior intercostal artery when 'the course of the latter is long'—that is, presumably, when it provides the intercostal artery to the second space. This finding was confirmed in the present series. He did not, however, observe the corollary—that when the course of the superior intercostal artery is short and the first aortic intercostal artery reaches the second space, the latter artery commonly provides branches to the lower part of the stellate or first thoracic ganglion, as described above.

An occasional contribution to the stellate ganglion along the ansa subclavia derived from the internal mammary artery, although admittedly inconstant, has not, to the writer's knowledge, been recorded previously.

SUMMARY

1. The only constant source of blood supply to the stellate ganglion is the superior intercostal artery, the nutrient vessels arising either directly or from its posterior rami and muscular branches passing behind the ganglion.

2. There is nearly always at least one accessory supply from other arteries in the vicinity, such as the costo-cervical trunk, deep cervical, thyro-cervical trunk and first aortic intercostal; less frequently the subclavian and the internal mammary arteries, the latter through a branch to the ansa subclavia. A notable exception is the vertebral artery which has not been seen taking part.

3. The ganglion is often situated within an arterial ring having cross-connexions passing behind the ganglion. From this vascular arrangement lateral, posterior and medial sets of ganglionic arteries arise.

4. There are free anastomoses between the ganglionic arteries both on the surface of the ganglion and within it; also between these vessels and those of the middle cervical and second thoracic ganglia.

5. From an anatomical point of view the blood supply to the ganglion appears to be very well assured.

I wish to express my thanks to Prof. G. A. G. Mitchell for his interest and most helpful advice. I would also gratefully acknowledge the valuable assistance I received from Mr F. L. Newell, who did the microradiography, and from Mr K. Pearson and Mr P. Howarth, who gave photographic and other technical help. A part of the expenses connected with the investigation has been defrayed by a departmental grant from the Medical Research Council.

REFERENCES

ANSEROW, N. I. (1925). Die Arterien des Rückenmarks, der Intervertebral-ganglien und der sympathischen Ganglien. *Anat. Ber.* **3**, 348–349.

BARTHOLDY, K. (1897). Die Arterien der Nerven. *Morph. Arb.* **7**, 393–458.

DELAMERE, G. & TANASESCO, J. C. (1906). Étude sur les artères du sympathique céphalique, cervical, thoracique et abdominal. *J. Anat., Paris*, **42**, 98–107.

DE SOUZA, O. M. (1932). Contribution à l'étude de la vascularisation du système nerveux organo-végétatif. *Ann. Anat. path. méd.-chir.* **9**, 975–997.

LOBSTEIN, J. F. (1823). *De Nervi Sympathetici humani fabrica usu et morbis*, pp. 67–68. Paris: Levrault.

PATTERSON, E. L. (1950). Sources of arterial blood supply to the superior and middle cervical sympathetic ganglia and the ganglion intermédiaire. *J. Anat., Lond.*, **84**, 329–341.

EXPERIMENTAL DETERMINATION OF RATE OF LONGITUDINAL BONE GROWTH

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Institute of Orthopaedics, British Postgraduate Medical Federation,
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In 1727, Steven Hales demonstrated experimentally, by measuring the leg-bone of a chick where 2 months earlier he had drilled holes to act as markers, that long bones grow by the addition of new tissues at the ends of the shaft, and not by general interstitial expansion. Duhamel (1742) and John Hunter (in about 1770) also performed this type of experiment† and Hunter, in the bones of young pigs which had been fed with madder, confirmed his conclusions by observing the terminal location of the new tissue formed under these circumstances. Later, the use of the microscope (Goodsir, 1845; Koelliker, 1852; Virchow, 1860) revealed that proliferation of cartilage, and the subsequent replacement of this tissue by bone, were the cellular processes on which longitudinal growth depends.

In a mammalian bone, the growing zone comprises the epiphysial cartilage plate and the bony tissue adjacent to its metaphysial surface. The general microscopic anatomy of this 'endochondral ossification' is now well known (Lacroix, 1949; Ham, 1950), but among different species of mammals there is wide variation in the detailed structure of the growing zone. Even within one species marked differences of histological pattern are found, according to the bone concerned and its stage of development. Although quantitative data have not so far been available, some, at least, of this structural variation is attributable to differences in growth rate. For this reason the purpose of the present investigation has been to determine directly the growth rate of the growing zone of the epiphysial plate at the lower end of the femur in the rat and the rabbit, and to use this information in interpreting the microscopic structure in the two species.

METHOD

Using Himalayan rabbits and rats of the Sprague-Dawley strain, data were collected on the growth occurring at the epiphysial plate at the lower end of the femur. After inserting a small metal marker into the femoral shaft, the increase in length of the distal part of the diaphysis was determined radiographically, pictures being taken every few days throughout the period studied (See Pl. 1, fig. 1).

The rabbits received a stock laboratory diet, including generous amounts of green vegetables. The rats received a commercial pellet diet‡ which contained vitamin and mineral supplements and which had a protein content of 21 %.

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* Part of this work was carried out during the tenure of a research fellowship in the Department of Pathology, Northwestern University Medical School, Chicago, Illinois, U.S.A.

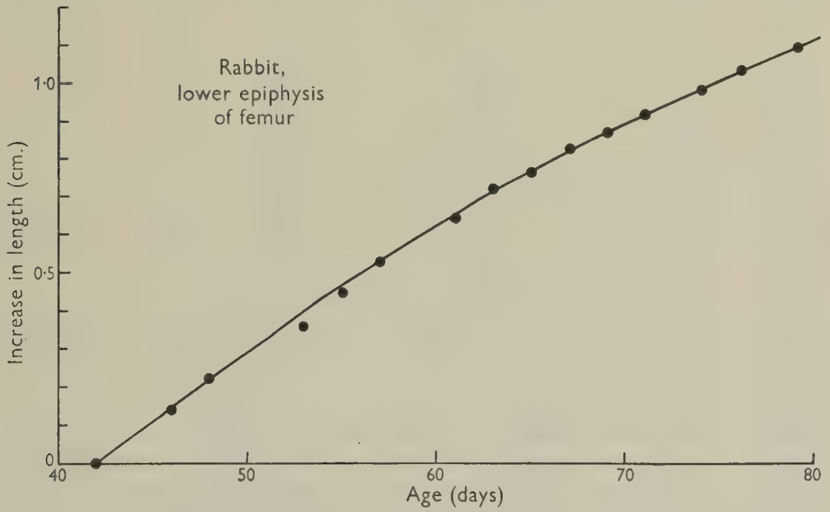
† Hunter's experimental specimen has been preserved, because of its historical interest, in the museum of the Royal College of Surgeons (See Dobson, 1948).

‡ 'Rockland' rat diet; from Rockland Farms, New City, New York (U.S.A.).

The radiographs were taken with the anaesthetized animal held in a standard position, its pelvis and legs strapped to a specially constructed frame. Fine-detail X-ray film was used, and the distance between the film and the X-ray tube was 60 in.*

Measurements on the films were made with dividers. It was convenient to measure the distance between the point of the marker and the surface of one of the femoral condyles, and then, by a second measurement on the same film, to correct this for any increase in size of the epiphysis itself.

The measurements for any individual animal usually fell on a smooth curve (Text-fig. 1) and such a record permits the calculation of the growth rate of the epiphysial plate during the period of the experiment.



Text-fig. 1. Rabbit. Longitudinal growth of the lower epiphysial plate of the femur.

RESULTS

Text-Fig. 1 shows the growth curve for the epiphysial plate at the lower end of the femur of a young male rabbit, approximately 6 weeks of age at the commencement of the experiment. The points plotted represent individual X-ray measurements. The original length of the measured segment of the femur is regarded as zero, only its subsequent increase being plotted.

Over the 34 days of the experiment, the rate of growth diminishes from 0·34 to 0·24 mm. per day, with an average value of 0·31 mm. per day.

Similar information for the rat is provided in Table 1, and represented graphically in Text-fig. 3. Data from six male rats of the Sprague-Dawley strain are tabulated, the graph being a smooth curve fitting the averaged values for these rats. At the commencement of the experiment the rats were 5 weeks old, and each weighed approximately 70 g. All rats were measured over a period of 5 weeks, and in three of them measurements were continued for a further 6 weeks. Over the initial 5-week period the average gain in weight for the rats was 5 g. per day. For the initial 5-week period the growth rate is 0·18 mm. per day, again being greater at the beginning than at the end of this time. Growth rates for the individual rats are 0·19, 0·18, 0·22, 0·16, 0·19 and 0·16 mm. per day respectively. The curve flattens considerably for the three rats allowed to survive longer, the growth rate over the subsequent 6-week period being approximately 0·04 mm. per day.

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* In a group of ten animals killed immediately after being X-rayed in this way, there was no 'parallax' error. The dimensions of the bones (direct measurement) and of their radiographs were identical.

Table 1

Age (days)	Length of measured segment of femur (in cm.)					
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
35	1·11	1·22	1·33	1·04	1·05	1·01
(0)	(0·00)	(0·00)	(0·00)	(0·00)	(0·00)	(0·00)
39	1·22	1·34	1·43	—	—	—
(4)	(0·11)	(0·12)	(0·10)	—	—	—
42	—	—	—	1·22	1·35	1·17
(7)	—	—	—	(0·18)	(0·30)	(0·16)
43	1·30	1·43	1·59	—	—	—
(8)	(0·19)	(0·21)	(0·26)	—	—	—
48	1·43	1·48	1·68	—	—	—
(13)	(0·32)	(0·26)	(0·35)	—	—	—
50	—	—	—	1·37	1·48	1·33
(15)	—	—	—	(0·33)	(0·43)	(0·32)
52	1·51	1·56	1·79	—	—	—
(17)	(0·40)	(0·34)	(0·46)	—	—	—
57	1·54	1·60	1·87	1·48	1·60	1·44
(22)	(0·43)	(0·38)	(0·54)	(0·44)	(0·55)	(0·43)
62	1·67	1·66	1·96	—	—	—
(27)	(0·56)	(0·54)	(0·63)	—	—	—
69	1·76	1·83	2·07	—	—	—
(34)	(0·65)	(0·61)	(0·74)	—	—	—
71	—	—	—	1·62	1·74	1·57
(36)	—	—	—	(0·58)	(0·69)	(0·56)
88	—	—	—	1·75	1·82	1·64
(53)	—	—	—	(0·71)	(0·77)	(0·63)
102	—	—	—	1·82	1·93	1·71
(67)	—	—	—	(0·78)	(0·88)	(0·70)
116	—	—	—	1·84	1·98	1·75
(81)	—	—	—	(0·80)	(0·93)	(0·74)

The figures in parentheses represent the time from the start of the experiment, and the increase in length of the measured segment in this period.

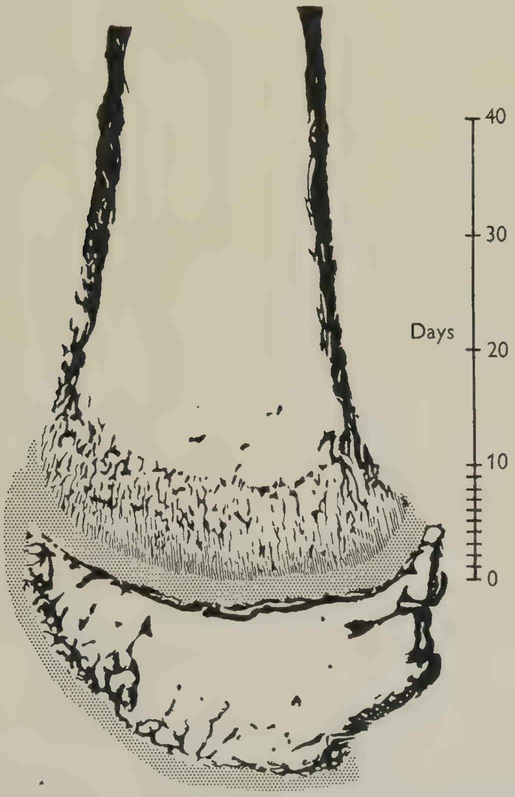
MICROSCOPIC STRUCTURE OF GROWTH ZONE

When the epiphysial plate at the lower end of the femur is examined microscopically in rats and rabbits of the age at which the above growth determinations were carried out, a considerable variation in structure is seen among the individuals of each group. The thickness of the epiphysial cartilage plate, and the number and arrangement of the metaphysial trabeculae, differ from animal to animal, whether early or late in the period studied. In contrast, measurements of growth rates for such a series of animals are relatively uniform, particularly when determined over periods longer than a few days. This histological variation suggests the possibility of some fairly rapid fluctuation in growth rate—and thus in structure—for any individual animal.

Because of this variation in histological structure the following descriptions and illustrations relate to bones which represent the 'mean' of a considerable number studied histologically at approximately the same age. It is obvious that the general appearance of the growing region of a bone in a microscopic section will depend on the plane in which the section has been cut. In the present study sections were cut in the sagittal plane, and passed through the longitudinal axis of the femoral shaft. If a section happened to deviate from this plane, it was not considered representative.

Rabbit

The general structure of the growing region of the lower femoral epiphysial plate in a rabbit 9 weeks old—corresponding to the middle of the period for which the growth determinations were made—is seen in Text-fig. 2.



Text-fig. 2. Rabbit (× 5). View of the lower end of the femur, with time scale calculated from the rate of growth.

The initial phase of the growth process consists of a proliferation and enlargement of the longitudinally arranged cells of the cartilage plate. The 'hypertrophic' cartilage is constantly eroded by vascular connective tissue from its metaphysial surface, and proliferating osteoblasts lay down bone on the surfaces of the remaining cartilage scaffolding.

Under conditions of uniform growth these processes are co-ordinated, and the rate of advance of the 'front' of vascularization and osteoblastic bone formation is the same as the growth rate of the cartilage of the epiphysial plate. Thus the thickness of the 'growing' cartilage plate remains constant, and the new bone laid down in its metaphysial surface is a measure of the rate of growth of the growing zone as a whole. In the present experiment this was found to be approximately 0·3 mm. per day.

Those bone trabeculae actually in process of formation are situated immediately adjacent to the surface of the epiphysial cartilage plate, while older trabeculae are left progressively farther away from this surface as growth continues. Assuming a uniform rate of growth, the 'age' of any bony structure can therefore be determined by measuring its distance from the advancing 'front'. In Text-fig. 2 and Pl. 1, fig. 2, the 'time-scale' at the side of the growing bone tissue shows this distance, the unit of measurement being the thickness of metaphysial bone laid down in 1 day (i.e. 0·3 mm.). Not only does the scale indicate the 'age' of bone trabeculae at its level, but it indicates the site of the growing surface of the epiphysial plate at the time of formation of these trabeculae.

By following histological changes in the bone trabeculae through the regions covered by the time scale, the approximate timing of the various cellular processes affecting them can be estimated.

In the zone of tissue 0·5 mm. wide adjacent to the advancing 'front'—that is for the first 36 hr. of their existence—the bone trabeculae are covered with proliferating osteoblasts and show deposition of lamellar bone on their surfaces (Pl. 1, fig. 3). Farther away from the 'front', continued osteoblastic activity increases the thickness of individual trabeculae, while extensive remodelling reduces their number and changes their originally longitudinal direction and parallel arrangement. In this zone, from about 0·5 to 2·5 mm. from the 'front' and thus covering a period of about 8½ days, there is evidence of both osteoblastic and osteoclastic activity, and the pattern of the reconstructed bone trabeculae presumably results from a balanced equilibrium between these two opposed processes.

Farther still, at a distance of about 2·5 mm. from the 'front', the metaphysial trabeculae terminate, osteoclasts being conspicuous at their ends, and presumably being the agents by which they are destroyed. This final dissolution usually takes place quite abruptly, a network of moderately thick trabeculae suddenly giving way to bone marrow which is almost entirely devoid of trabeculae. The narrowness of the zone of conspicuous osteoclasis, which is no more than 0·2–0·3 mm. in width, shows that this is a rapid process, no more than 16–24 hr. being taken for the complete destruction of bone trabeculae as they enter this region. In addition to the osteoclasts engaged in the erosion of bone trabeculae, the zone of destruction contains additional cells of this type which are not related to bone trabeculae and which appear to have persisted after the disappearance of these structures.

From this description, it can be seen that the average 'life-history' for an individual bone trabecula of the growing region is as follows: an initial period of approximately 36 hr., when predominantly osteoblastic activity occurs; then progressive remodelling involving balanced osteoblastic and osteoclastic activity, continuing for about 8–9 days; finally abrupt osteoclastic destruction, attaining completion in less than 24 hr. This description is put forward as an 'average' for the femoral epiphysis of a rabbit at the age of 9 weeks, and individual animals may show some transient departure from it.

In the present investigation, the proliferation and maturation of cells in the cartilage plate have not been considered. It should be remembered that, although the cartilage plate has the same overall rate of growth as the bone tissue, the time-scale shown for the bone cannot be applied to the interpretation of distances within the growing cartilage.

Rat

The general sequence of events in the growth of the lower femoral epiphysial plate of the rat is similar to that described for the rabbit, although there are considerable differences in details. Text-fig. 4 shows the general appearance of this region in a rat 50 days of age, at the centre of the initial 5-week period for which growth determinations are shown in Text-fig. 3, and therefore assumed to be growing at approximately 0·18 mm. per day. Some rats show a thicker proliferating zone of the epiphysial cartilage and a more regular pattern of newly formed bone trabeculae adjacent to it, and this 'optimal' appearance is the one usually illustrated and described in the literature.

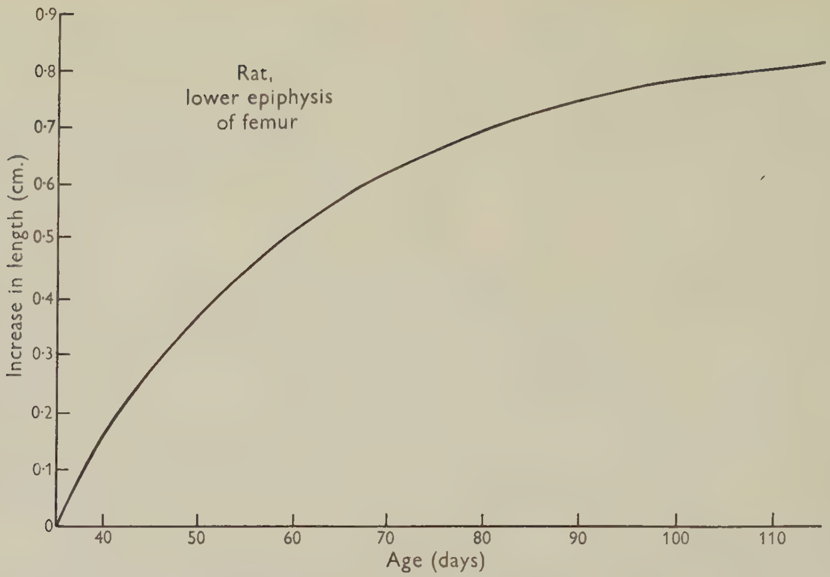
The apices of the metaphysial trabeculae show osteoblastic proliferation and lamellar bone deposition, but, in the rat femur illustrated (Pl. 1, fig. 4) evidence of remodelling of the trabeculae is present almost from the start, and is active at 0·2 mm. from the 'front'—i.e. about 24 hr. after the initial formation of the trabeculae. In most of the rat femora studied, remodelling of metaphysial trabeculae changes gradually to osteoclastic resorption, the abrupt line of dissolution described in the rabbit being absent. At a distance of 0·7 mm. (equivalent to 4 days) from the 'front', trabeculae are reduced in thickness and in number, but not until a distance of 3·4 mm. (i.e. 19 days) are they completely absent. In accord with this finding, osteoclasts are not concentrated in a narrow zone, but are fairly evenly scattered over the surfaces of bone trabeculae in the broader area of their dissolution.

DISCUSSION

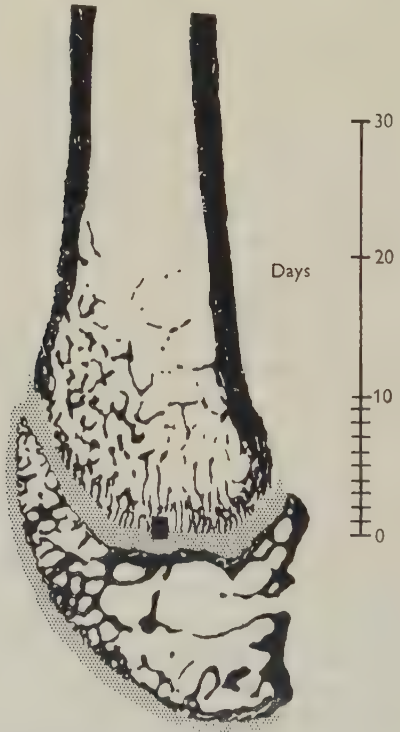
The literature contains occasional items of information from which the rate of longitudinal bone growth in various species can be estimated, but no comparisons have been made between such growth rates and the microscopic structure of the corresponding bones.

In the rabbit, for instance, some experiments of Dubreuil (1913) are available, where the tibia was X-rayed at intervals after the insertion of metallic markers into the shaft. Measurements of this author's illustrations give a value of approximately 0·4 mm. per day for the rate of growth of the proximal epiphysial plate of the tibia during the early period of active growth. No figures for the femur are given.

In the rat, from measurements made on bones at various stages in their development (Donaldson, 1924) an approximate figure of 0·2 mm. per day can be obtained for the rate of longitudinal growth of the whole femur (i.e. the combined rates for the upper and lower ends) between the ages of 50 and 90 days. Similar figures provided by Ray, Simpson, Li, Asling & Evans (1950) for the femur length in normal rats at 30 and 70 days of age, give a figure of 0·17–0·19 mm. per day for the



Text-fig. 3. Rat. Longitudinal growth of the lower epiphysial plate of the femur.



Text-fig. 4. Rat (× 10). View of the lower end of femur, with time scale calculated from the rate of growth. The black area indicates the location of Pl. 1, fig. 4.

growth rate of the whole bone over this period. In their studies with 'bone-seeking' radioactive isotopes, Leblond, Wilkinson, Belanger & Robichon (1950), using autoradiographs of bones from growing animals killed at varying intervals after the injection of such isotopes, were able to identify—from the region of most intense radioactivity—the site of the zone of calcification of the epiphysial plate at the time of injection. The depth of new bone formed subsequently could thus be determined, and in young rats measurements suggested a growth rate of approximately 0·15 mm. per day for the upper end of the tibia, and 0·10 mm. per day for the upper end of the humerus. No figures for the femur were reported.

In the pig, the data of Payton (1932), who experimented with madder feeding, allow the growth rates of a variety of epiphysial plates to be estimated. Values range from about 0·2 to 0·5 mm. per day at 80 days of age, and from 0·05 to 0·18 mm. per day at 150 days. In the goat, Bisgard & Bisgard (1935) inserted metallic bone markers in order to determine the relative amount each epiphysial plate contributes to the length of a bone. Their figures give a rate of 0·36 mm. per day for the lower epiphysial plate of the ulna between 4 and 32 days of age, and of 0·1 mm. per day for the same structure at 180 days.

In man, the growth rates of the various epiphysial plates of the skeleton have been studied in some detail, although again there has been no attempt to correlate known growth rates with the histological structure of the tissues concerned. Especially in the lower limb, a knowledge of epiphysial growth rates is of clinical importance in the prediction of the relative limb-shortening that will follow the operative arrest of growth of any particular epiphysial plate (Blount & Zeier, 1952; Green & Anderson, 1947; Gill & Abbott, 1942). From the radiographic data of Gill & Abbott (1942) the rate of growth of the lower femoral epiphysial plate between the ages of 5 and 13 years is estimated at approximately 1·25 cm. per year (i.e. as little as 0·035 mm. per day). The great difference between this rate of growth for an epiphysial plate in man, on the one hand, and those rates determined in small laboratory animals, on the other, should be remembered when the histological structures of the corresponding growing regions are compared.

SUMMARY

Using young rabbits and rats, the rate of growth of the epiphysial cartilage plate at the lower end of the femur has been determined radiographically. A figure of 0·31 mm. per day was obtained for the rabbit (age 9 weeks), and 0·18 mm. per day for the rat (age 50 days).

This information is used in interpreting the microscopic structure of the growing region of the bone in each species, and in determining the duration of each of the cellular processes concerned in the formation and maturation of bone trabeculae in this situation.

The relative duration of the various cellular processes varies from species to species, and this factor, in addition to variation in absolute growth rates, is responsible for the differences in the microscopic structure of the growing region in the two species studied.

REFERENCES

Bisgard, J. D. & Bisgard, M. E. (1935). Longitudinal growth of long bones. *Arch. Surg., Chicago*, **31**, 568–578.

Blount, W. P. & Zeier, F. (1952). Control of bone length. *J. Amer. Med. Ass.* **148**, 451–457.

Dobson, J. (1948). Pioneers of osteogeny. John Hunter. *J. Bone Jt Surg.* **30 B**, 361–364.

Donaldson, H. H. (1924). The rat; data and reference tables for the Albino rat and the Norway rat. 2nd ed. revised. *Mem. Wistar Inst. Anat.* No. 6.

Dubreuil, G. (1913). La croissance des os des Mammifères. III. L'accroissement interstitial n'existe pas dans les os longs. *C.R. Soc. Biol., Paris*, **74**, 935–937.

Duhamel, H. L. (1742). Sur le développement et la crûe des os des animaux. *Mém. Acad. Roy. Sci.* **55**, 354–370.

Gill, G. G. & Abbott, L. C. (1942). Practical method of predicting the growth of the femur and tibia in the child. *Arch. Surg., Chicago*, **45**, 286–315.

Goodsir, J. (1845). The structure and economy of bone. In *Anatomical and Pathological Observations*, p. 64 by J. Goodsir and H. D. S. Goodsir. Edinburgh: M. Macphail.

Green, W. T. & Anderson, M. (1947). Experiences with epiphysial arrest in correcting discrepancies in length of the lower extremities in infantile paralysis. *J. Bone Jt Surg.* **29**, 659–675.

Hales, S. (1727). *Statistical Essays*, p. 339. London: W. Innys.

Ham, A. W. (1950). *Histology*. Philadelphia: J. B. Lippincott.

Hunter, J. See 'Experiments and observations on the growth of bones, from the papers of the late Mr Hunter' in *The Works of John Hunter*, vol. 4, ed. by James F. Palmer (1837). London: Longmans Green. See also *Menders of the Maimed*, chap. xiv by A. Keith (1919), London: Oxford University Press.

Koelliker, A. (1852). *Handbuch der Gewebelehre des Menschen*. Leipzig: W. Engelmann.

Lacroix, P. (1949). *L'organisation des os*. Paris: Masson & Cie

Leblond, C. P., Wilkinson, G. W., Bélanger, L. F. & Robichon, J. (1950). Radio-autographic visualization of bone formation in the rat. *Amer. J. Anat.* **86**, 289–341.

Payton, C. G. (1932). The growth in length of the long bones in the madder-fed pig. *J. Anat., Lond.*, **66**, 414–425.

Ray, R. D., Simpson, Miriam E., Li, C. H., Asling, C. W. & Evans, H. M. (1950). Effects of the pituitary growth hormone and of thyroxin on growth and differentiation of the skeleton of the rat thyroidectomized at birth. *Amer. J. Anat.* **86**, 479–516.

Virchow, R. (1860). Normal and pathological new-formation. In *Cellular Pathology*, translated from the 2nd German edition, 1858, by Frank Chance. London: J. Churchill.

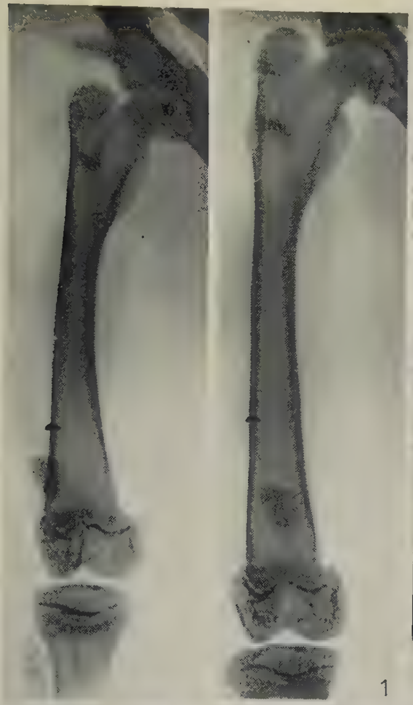
EXPLANATION OF PLATE

Fig. 1. X-rays of rabbit femur, before and after an interval of 10 days. The marker allows the longitudinal growth of the lower epiphysial plate during this period to be determined.

Fig. 2. Rabbit (× 30). View of the epiphysial plate and metaphysial bone trabeculae shown in Text-fig. 2. The time scale indicates the 'age' of any bone trabeculae, as measured from the growing surface of the epiphysial plate.

Fig. 3. Rabbit (× 122). Growing cartilage and newly formed bone trabeculae from Pl. 1, fig. 2.

Fig. 4. Rat (× 123). Growing cartilage and newly formed bone trabeculae from the area indicated in Text-fig. 4.



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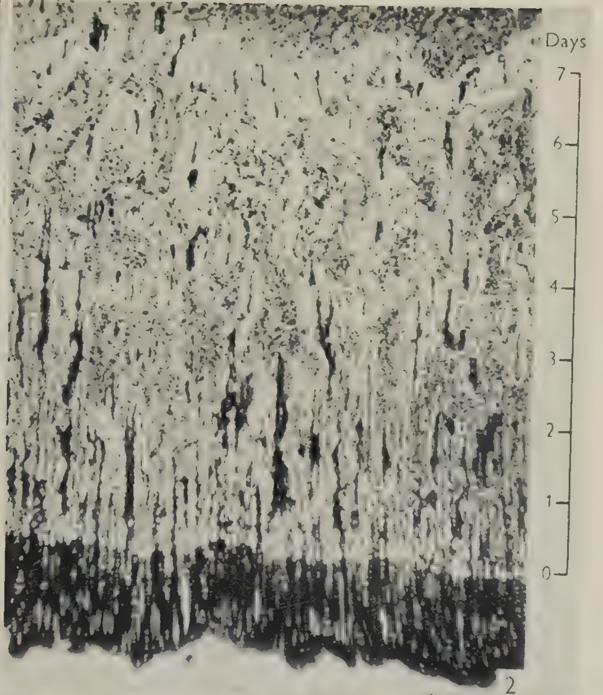

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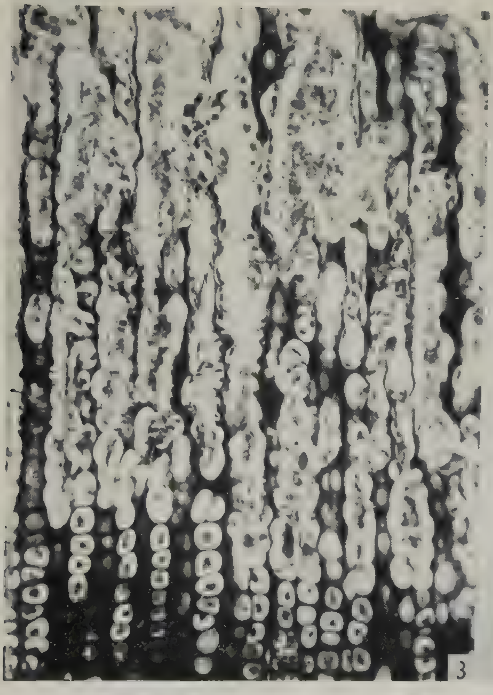

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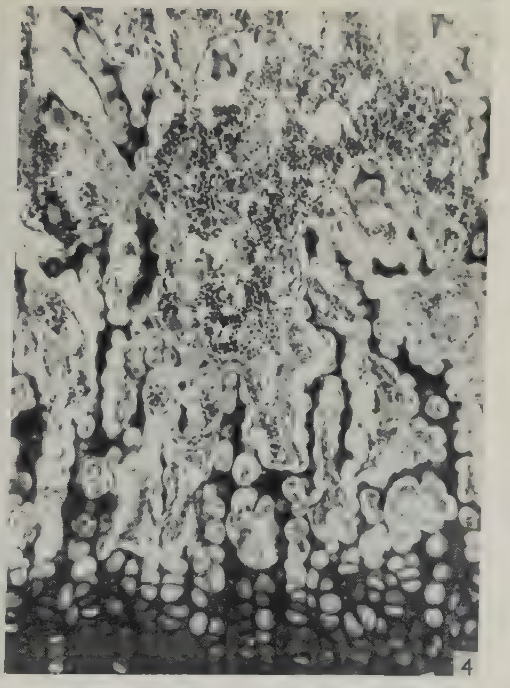

4

SISSONS—Experimental determination of rate of longitudinal bone growth

ALKALINE PHOSPHATASE IN THE EPITHELIAL FREE BORDER OF THE EXPLANTED EMBRYONIC DUODENUM

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INTRODUCTION

Since Gomori's (1941) original report upon the presence of alkaline phosphatase in the free borders of the epithelial cells lining the duodenum, our knowledge of the enzyme has been extended in some directions. For instance, it is now known that a group of enzymes are located in the free border which differ in their pH optima and substrate specificity (Dempsey & Deane, 1946). The histochemical technique used in the demonstration of this site of phosphatase activity has itself been criticized, and the validity of its results has been the subject of several investigations (Leduc & Dempsey, 1951; Martin & Jacoby, 1951; Moog, 1950, 1951; Moe, 1952).

In other directions little progress has been made. The precise stage of embryonic development at which phosphatase can first be demonstrated in the free borders is uncertain. Hébert (1950), working with the fowl, was unable to detect enzyme until after hatching; but Moog's (1950) careful investigations revealed a positive reaction on the 17th day of incubation. There is as yet little evidence to show what factors govern the first appearance of the enzyme in the embryonic duodenum; Hébert (1950) has claimed that the suprarenal cortex is involved. This hypothesis has been tested directly in the experiments described below. Samples of embryonic duodenum have been explanted in tissue culture where they were maintained for varying periods apart from the normal humoral stimuli; the phosphatase content of the free borders of their epithelial cells was then investigated histochemically.

MATERIAL AND METHODS

Embryonic fowl tissues were used throughout; the explants were prepared as follows. The duodenal loops were excised from embryos of 14 or 15 days' incubation, that is, at a stage of incubation 3 or 2 days earlier than that at which Moog (1950) could first demonstrate enzyme in the free border. The loops were transferred to Tyrode solution, freed of the adherent pancreas, and transected into a number of short lengths, which were split open by a longitudinal incision. The resulting sheets of tissue soon rolled up to form incomplete tubes with the mucosal surface outward, and a long axis at right angles to the original gut lumen. One or two random samples from each donor were fixed at once in 85 % alcohol chilled to −5° C. The remainder, generally around six for each donor, were explanted *in vitro*.

A modified form of Fell's watch-glass technique was employed (Fell & Robison, 1929) for the tissue culture. The watch-glasses containing the explants upon their nutrient clots were placed inside Petri dishes with loose-fitting lids, and these were stacked inside a glass desiccator vessel. The well of the vessel contained cotton-wool moistened to humidify the atmosphere. The constitution of the latter could be varied at will and consisted usually of 5 % CO_2 in O_2.

The culture medium consisted of a plasma clot: it was found that best results were obtained by diluting the plasma with an equal volume of Tyrode solution. The diluted plasma was clotted by the addition of an equal volume either of fresh embryo extract or of dried thrombin (Maw) dissolved in Tyrode solution. Penicillin (crystalline sodium salt, Glaxo) was added to the culture medium in a final concentration of 50 i.u./ml. of medium without noticeable adverse effect.

The explants from each donor were incubated together in the same watch-glass and in each experiment from two to six donors were used. After an incubation period of 2–14 days, the Petri dishes were removed from the desiccator, the living explants scrutinized, and then fixed in chilled 85 % alcohol and sectioned in paraffin.

For the histochemical demonstration of sites of alkaline phosphatase activity, Kabat & Furth's (1941) modification of the original Gomori method was employed; calcium phosphate deposits were, however, made visible by the cobalt sulphide technique. As a routine, sections were incubated in the substrate bath at 39° C. for periods of $\frac{1}{2}$ and 1 hr. Control sections were treated in the accepted manner, either by heating to 90° for 1 min. prior to incubation in the substrate bath, or by the use of a substrate bath from which glycerophosphate had been omitted. Sections were also stained with haematoxylin and eosin.

RESULTS

(*a*) *Observations on living explants*

Explants could be examined only at the start and finish of the experiments. There was vigorous liquefaction of the plasma clot; after 3 days, the entire contents of the watch-glasses were often fluid. The explants rapidly became larger and by the 3rd day had usually become spherical or cylindrical masses often with tapered ends. Encircling the entire outer surface were innumerable rows of parallel lines, the primordial villous ridges. As time went by these continued to mature. They became higher and the continuous ridges were transformed into rows of individual finger-like processes.

The ridges or digitations afforded a rough index of the condition of the explants. In the best, there were tall projections; intermediate grades possessed fewer and shorter elevations, whilst at the other end of the scale some explants presented a smooth surface. The last mentioned were also generally opaque and white, and were discarded. From time to time small transparent bubble-like excrescences grew out from the surfaces of the explants. These were produced by uncontrolled growth of the epithelium which formed cysts.

The site of the longitudinal incision could be seen as a puckered depression; it persisted until around the 7th day of incubation, although sections showed that it had healed by the 2nd day. The explants undoubtedly increased rapidly in size, especially in the early stages of incubation, but unfortunately it was not possible to express the growth quantitatively. As will be described below, fluid uptake and storage is believed to have been an important factor in their apparent growth.

The epithelium was held firmly in place in the healthy explants; it could not be detached by microsurgical exploration. Detached epithelium was seen floating around only in the case of unhealthy explants.

(*b*) *Observations on fixed material*

Altogether approximately 100 explants were sectioned, the ribbon of sections of each explant being sampled at different points on its length. The results are arranged below in chronological order. The validity of the observations upon sites of phosphatase activity was carefully checked throughout by means of control sections as described above. In the interests of brevity they will not be mentioned again below.

(1) *Two days' incubation.* Six explants were fixed. As can be seen in Pl. 1, figs. 1 and 2, the villi were well preserved. The longitudinal incision, the original site of which may still be discerned, had healed, the surface being epithelialized.

The epithelium on the whole seemed healthy, and the cells had become taller than those of the control samples (that is, prepared lengths of duodenum from the same donor fixed without explantation). This was no doubt a reflexion of their continuing differentiation. In places, however, the epithelium had begun to alter. The proximal poles of the cells were loaded with small vacuoles which seemed to have become confluent here and there, producing large spaces in the proximal cytoplasm. Especially at the tips of the villi the entire proximal zone seemed to have been filled, probably with fluid, and the cytoplasm was reduced to a thin central infranuclear stalk. In places, the distal cytoplasm as well was loaded with small droplets (Pl. 1, fig. 3).

The connective tissue beneath the epithelium was in places distended as if by intercellular fluid, its cells being widely separated. In the deeper layers there was also some dilation of vessels in which erythrocytes could still occasionally be distinguished (Pl. 1, fig. 4).

Alkaline phosphatase could be demonstrated in the nuclei and cytoplasm of the stromal connective tissue and in the nuclei and free borders of the epithelial cells. Sometimes the border reaction was confined largely to the sides and bases of the villi; sometimes their tips also were positive (Pl. 1, fig. 5).

(2) *Three days' incubation.* Twenty explants were examined. They had maintained their general organization well (Pl. 1, fig. 6). Compared with controls, the villi had become longer and the epithelial cells taller (Pl. 1, figs. 7, 8). In places they showed further signs of the changes described in the 2-day specimens. Droplets of varying size had continued to accumulate in their proximal poles, especially near the tips of the villi (Pl. 1, fig. 9). In many explants the deeper portion of the mucosa contained a series of dilated cavernous spaces often almost confluent in the deeper zones (Pl. 1, figs. 6, 7). Remains of erythrocytes could be distinguished within. The subepithelial connective tissue in the cores of the villi sometimes also possessed dilated vascular spaces (Pl. 1, fig. 10); sometimes the connective tissue cells themselves were widely separated as if intercellular fluid had been present (Pl. 1, fig. 11). Observations suggested that the epithelial cells had absorbed fluid

from their distal borders; that it was secreted from the proximal pole, where a portion was retained, distending the proximal cytoplasm; that a proportion was transported into the stroma, where some entered the vascular channels which became dilated in the absence of the normal pathways of escape, whilst the remainder was retained between the connective tissue cells.

Phosphatase could be demonstrated in control sections (Pl. 1, fig. 12). It was present in the stroma cells, and the epithelial nuclei and free borders. The same distribution was seen in sections from many explants (Pl. 2, fig. 13); some, however, lacked demonstrable enzyme.

Omission of embryo extract and its substitution by thrombin in the culture medium had no adverse effect upon the mucosa, although the deeper tissues were often necrotic. Enzyme was demonstrable as before (Pl. 2, fig. 14). The control sample for this particular explant is shown in Pl. 2, fig. 15, and the free border can be seen to have had a strongly positive reaction.

(3) *Six days' incubation.* Twenty explants were available; they were maintained on extract clots, and were subcultured to fresh medium on the 3rd day.

Regressive changes had affected the epithelium in many instances. It had become irregular, with local cauliflower-like outgrowths and was often stratified. In other explants, however, it remained in good condition, although the deeper tissues were often degenerate. Villous projections were still to be seen (Pl. 2, figs. 16, 17).

The 'loading' of the epithelial cells, early stages of which were described above, had advanced. Adjacent cells were often completely separated along their lateral boundaries whilst the proximal infranuclear cytoplasm was compressed into a thin tail. However, the free borders remained intact from cell to cell and presented an uninterrupted pellide. Sometimes the epithelium was completely disrupted as if burst by accumulating fluid (Pl. 2, fig. 18).

The sections stained to demonstrate sites of phosphatase activity showed several features of interest. First, there was often a positive border reaction (Pl. 2, figs. 17, 19); secondly, positively reacting granules could be distinguished in the distal cytoplasm (Pl. 2, fig. 19); and thirdly, the reaction of the stromal connective tissue cells was weak and sometimes negative. A fourth observation was that phosphatase could still be demonstrated in the free border, overlying 'loaded' cells, even when actual cell disruption had occurred (Pl. 2, fig. 20).

Control sections showed shorter villi; the free borders of their epithelial cells gave a positive reaction (Pl. 3, fig. 21). In one or two explants examined at this time there seemed to be evidence that morphological and chemical differentiation did not necessarily occur together. Epithelial cells from two sister explants are shown in Pl. 2, figs. 19 and Pl. 3, fig. 22. These were derived from the same donor and therefore had been maintained in the same watch-glass, fixed and embedded, sectioned, and stained together. The two samples of epithelium resemble each other fairly closely but only one showed a positive border reaction (Pl. 2, fig. 19).

(4) *Seven days' incubation.* Twenty explants were fixed. Some possessed long villi but in most the epithelium had regressed. It was well preserved however, in places, and gave a positive border reaction (Pl. 3, figs. 23, 24). The enzyme distribution in the free borders was uneven and patchy in most explants at this time (Pl. 3,

figs. 24, 25). The control sample for this latter explant had a strongly positive border reaction.

(5) *Eight and nine days' incubation.* Sections of fifteen explants were available. Villi were still to be seen in some cases, but were not the rule. Usually the explant surface was smooth or had short rounded projections. The free border still gave a positive reaction in places and there were often positively stained granules to be seen in the distal cytoplasm. On the whole the border reaction had declined.

(6) *Fourteen days' incubation.* Fifteen explants were available. They had been subcultured twice. The villi were reduced in height and the central zones necrotic (Pl. 3, fig. 26). A positive, though much 'moth-eaten' border reaction was still to be obtained (Pl. 3, fig. 23).

Eight further explants were fixed in Bouin's solution, in order to determine whether the epithelial 'loading' encountered throughout the experiments was merely a shrinkage artifact produced by alcohol fixation. 'Loading' was still apparent in the Bouin-fixed material (Pl. 3, fig. 28).

DISCUSSION

In the past, much valuable information has been gained concerning the ability of various organs and body regions to differentiate in a self-regulatory manner in tissue culture (Fell, 1951). The criterion has been of histological appearance. That little work has so far been carried out upon the histochemical aspects of differentiation *in vitro* is a reflexion of the fact that reliable histochemical techniques are of comparatively new invention. Fell & Robison (1929), however, were able to demonstrate the occurrence of alkaline phosphatase in explants of limb-bud cartilage by biochemical methods.

The enzyme has been demonstrated in the nuclei of cells in tissue culture on several occasions (Willmer, 1942; Chèvremont & Firket, 1949). The free border of the duodenal epithelial cell, however, like that of the epithelial cell of the convoluted tubule of the kidney, the hypertrophic cartilage cell and the osteoblast, may be considered as a specific site for enzyme localization. There seem to be no previous reports of the histochemical demonstration of phosphatase in such sites in tissue maintained *in vitro*.

Fischer's (1922) studies upon 'intestinal organisms' in tissue culture were based upon explants taken from much older donors; they were of 20–21 days' incubation. The explants were derived from the 'small intestine', and it is impossible to tell whether any came from the duodenum. They were grown in an atmosphere of air. Fischer encountered (and illustrated) changes in the epithelium similar to those described above but did not, apparently, regard them as of any particular functional significance. On the whole the condition of the cultures in the present experiments seems to have been better. The villi were longer than those illustrated by Fischer in spite of the initial advantage in height possessed by his older explants, and the general preservation of the tissues better. This is probably due to the use of O_2 with 5% CO_2. Others have maintained cultures of the embryonic small intestine (Bisgelie, 1932), using continuous perfusion techniques in some instances (de Jong & de Haan, 1943; Pothoven, 1936).

The longest experimental period employed in the present series was 14 days, at the end of which alkaline phosphatase was still demonstrable in places in the free borders. It is necessary to discuss whether the enzyme was simply carried over in the free borders at the start of the experiments, there to persist during the period of life *in vitro*, or whether it was actually elaborated, wholly or partly, during the period of explantation.

The fact that enzyme was clearly demonstrable in the free borders of the majority of the control samples indicates that, in some cases at least, the explanted samples also must have possessed phosphatase in their free borders at the start of the experiment. This enzyme may well have persisted *in situ* throughout the experimental period. The normal life span of enzyme molecules in the body is unknown. Nor is it certain whether in the normal course of events all or any of the free border phosphatase migrates to the gut lumen. If this were known to happen regularly, it would afford an argument against the view that the explant phosphatase remained unaltered during life *in vitro*; but Moog's (1950) observations suggest that there is normally no free enzyme in the gut lumen, and that it reaches the lumen only as a result of mechanical transport thither in desquamating, disintegrating cells. If enzyme elaboration did not occur in the explants then it is necessary to accept that molecules of phosphatase may endure for at least 14 days.

On the other hand, the fact that the border reaction was more substantial in explants maintained for the shorter periods such as 2 or 3 days than in control samples does suggest that some 'enzymogenesis' (Moog, 1950) occurred *in vitro*, and the adrenal cortex must play an unimportant part in its appearance. The gradual weakening and patchiness of the border reaction observed in the later stages suggests either that enzymogenesis is weakened with time, or that the enzyme, though elaborated by the explants, was not concentrated and held at the free border. Concentration of the enzyme at the free border must be the result of two processes which may well be independent. First, there must be production of enzyme, and secondly, there must be some special chemical or physical differentiation of the free border having a particular affinity for the enzyme. Moog & Wenger (1952) have recently suggested that a polysaccharide may act in this way. The latter factor may have failed in the case of explants possessing a negative reaction at the free border. It is possible that careful morphological investigation might have revealed some deficiency of development of the striated border. The method of choice would be the electron microscope as used by Granger & Baker (1950); but the alcohol fixation used in the present experiments precluded a serious examination of the material from this point of view.

The tenacity with which the enzyme is held in the free border is indicated by its failure to diffuse or leach out from the severely damaged cells illustrated in Pl. 3, fig. 24. This behaviour of living tissue is in sharp contrast to the rapid loss of enzyme from the epithelial free border in tissue sections, described first by Moog (1950) and later by Moe (1952), which occurs during prolonged incubation in the substrate bath.

Embryo extract, a saline extract of whole embryos, is rich in phosphatase. Its presence in the nutrient medium might, conceivably, have been followed by adsorption of enzyme on to selective sites such as the free border. Martin & Jacoby

(1951) have demonstrated the operation of a similar occurrence with superimposed paraffin sections of organs rich and poor in the enzyme; Leduc & Dempsey (1951) have shown that nuclei and cytoplasm, in sections, can adsorb enzyme from a solution at pH 7·0. However, sections from explants maintained in an extract-free medium, in which thrombin was present, presented an exactly comparable picture of enzyme distribution even after 8 days *in vitro*. Adsorption cannot therefore have had much effect upon the distribution but may, as a remote possibility, have influenced the intensity of the reaction. It is a little surprising that explants maintained in thrombin clots were able to adapt themselves better to life *in vitro* than those grown in extract clots. Their villi were longer; their epithelium was better preserved; phosphatase was distributed more evenly on the epithelial surface.

The 'loading' of epithelium and stroma, together with the formation of dilated cavernous spaces, seems to indicate active absorption and storage of fluid by the explants, and this was undoubtedly an important factor in their very rapid increase of size. It is hoped to test this hypothesis directly by means of coloured and radioactive tracer substances.

Moog (1950) has described a gradual weakening of the stromal reaction, with time, accompanied by concentration of the enzyme in the free borders, more or less complete by the 17th day of incubation. The epithelial cells also increased steadily in height. Similar changes were apparent in the explants and no doubt reflected a continuation of the normal sequences of differentiation.

The stage of incubation of the fowl embryo at which Moog was first able to demonstrate phosphatase in the duodenal free border was the 17th day. It was surprising, therefore, to find an unmistakable positive zone, at the distal margins of the cells in embryos of 14 days' incubation (Pl. 1, fig. 12; Pl. 2, fig. 15). This result was unexpected. The histochemical method employed in our experiments entailed the use of magnesium ions, which are recognized activators of phosphatase, in the substrate bath; on the other hand, Moog used the substrate described by Gomori (1941) which lacks magnesium so that her technique was probably somewhat less sensitive.

SUMMARY

1. The duodenal loops were excised from fowl embryos of 14 or 15 days' incubation and transected into a number of short lengths. These were split open longitudinally. Some were explanted *in vitro*, whilst others were fixed at once in chilled alcohol.

2. Diluted fowl plasma was clotted by the addition of either embryo extract or thrombin solution and the resulting mixture used for the nutrient medium. An atmosphere of 5 % CO_2 in O_2 was provided for the cultures which were grown in watch-glasses.

3. After periods varying from 2 to 4 days the explants were examined and then fixed in chilled alcohol. They were embedded in wax and serial sections cut.

4. Sections were treated by Kabat & Furth's (1941) modification of the Gomori technique for the demonstration of sites of alkaline phosphatase activity. Others were stained with haematoxylin and eosin. Control sections, obtained from samples of duodenum fixed without explanation, were similarly treated.

5. The explants increased in size and the villi, on their outer surfaces, grew higher and were well-preserved. Considerable absorption and retention of fluid is thought to have occurred.

6. Alkaline phosphatase could be demonstrated in the free borders of the explanted epithelial cells even after 14 days *in vitro*; the distribution of enzyme was identical irrespective of whether embryo extract or thrombin had been used in the culture medium. Explants did better with the latter than with the former.

7. Comparison of sections of control samples, fixed before explantation, with those of samples grown for 2–6 days *in vitro*, showed the border reaction to be more substantial after explantation.

8. A positive border reaction was obtained with control samples of 14 days' incubation. This conflicts with Moog's (1950) report that the border reaction in the developing fowl embryo is not positive until the 17th day.

9. The question whether enzyme was elaborated *in vitro*, or merely persisted, is discussed.

REFERENCES

Bisgelie, V. (1932). Studi sui tessuti espiantati. (2) L'attiva peristaltica degli espiantati di intestino embrionale. (3) La modalita di accrescimento ed i caratteri strutturali delle culture di intestino embrionale di pollo. *Arch. exp. Zellforsch.* **12**, 86–124.

Chèvremont, H. & Firket, H. (1949). Phosphatase alcaline dans les cellules cultivées *in vitro*; ses rapports avec la croissance. *Arch. Biol., Paris*, **60**, 441–472.

Dempsey, E. W. & Deane, H. W. (1946). The cytological localization, substrate specificity, and pH optima of phosphatases in the duodenum of the mouse. *J. cell. comp. Physiol.* **27**, 159–179.

de Jong, B. J. & de Haan, J. (1943). Organ and tissue differentiation in perfused cultures of explants from the oesophagus, stomach, and trachea complex of young chicken embryos. *Acta neerl. morph.* **5**, 26–81.

Fell, Honor B. (1951). In *Cytology and Cell Physiology*, chapter x, pp. 419–443. Ed. by G. H. Bourne. Oxford: Clarendon Press.

Fell, Honor B. & Robison, R. (1929). The growth, development, and phosphatase activity of embryonic avian femora and limb-buds cultivated *in vitro*. *Biochem. J.* **23**, 767–784.

Fischer, A. (1922). Cultures of organised tissues. *J. exp. Med.* **36**, 393–397.

Gomori, G. (1941). The distribution of phosphatase in normal organs and tissues. *J. cell comp. Physiol.* **17**, 71–83.

Granger, Barbara & Baker, R. F. (1950). Electron microscope investigation of the striated border of intestinal epithelium. *Anat. Rec.* **107**, 423–441.

Hébert, Simone (1950). Les phosphatases alcalines de l'intestin. Étude histochimique expérimentale. *Arch. Biol., Paris*, **61**, 237–289.

Kabat, E. A. & Furth, J. (1941). A histochemical study of the distribution of alkaline phosphatase in various normal and neoplastic tissues. *Amer. J. Path.* **17**, 303–318.

Leduc, Elizabeth H. & Dempsey, E. W. (1951). Activation and diffusion as factors influencing the reliability of the histochemical method for alkaline phosphatase. *J. Anat., Lond.*, **85**, 305–315.

Martin, B. F. & Jacoby, F. (1951). Diffusion phenomenon complicating the histochemical reaction for alkaline phosphatase. *J. Anat., Lond.*, **83**, 351–363.

Moe, H. (1952). On the histochemical demonstration of alkaline glycerophosphatase and its dependence on the incubation time. *Anat. Rec.* **112**, 217–235.

Moog, Florence (1950). The functional differentiation of the small intestine. I. The accumulation of alkaline phosphomonoesterase in the duodenum of the chick. *J. exp. Zool.* **115**, 109–129.

Moog, Florence (1951). The functional differentiation of the small intestine. II. The differentiation of alkaline phosphomonoesterase in the duodenum of the mouse. *J. exp. Zool.* **118**, 187–207.

Moog, F. & Wenger, E. L. (1952). The occurrences of a neutral mucopolysaccharide at sites of high alkaline phosphatase activity. *Amer. J. Anat.* **90**, 339–377.

Pothoven, W. J. (1936). *Her Kweken van Weefsels in Doorstroomde cultures*, pp. 1–133. Drukkerij gebroeders hoitsema groningen.

Willmer, E. N. (1942). The localization of phosphatase in cells in tissue cultures. *J. exp. Biol.* **19**, 11–13.

EXPLANATION OF PLATES

Plate 1

Fig. 1. Two-day explant, haematoxylin and eosin. The incision, whose original site is visible on the right, has healed. × 42.

Fig. 2. Two-day explant, haematoxylin and eosin. The healed incision is visible on the left. The darkly stained circular mass within was a portion of pancreas accidentally included in the explant. × 52.

Fig. 3. Two-day explant, haematoxylin and eosin. A villus tip is covered by epithelial cells which show early signs of loading with fluid. The infranuclear cytoplasm is reduced to a thin stalk and contains small vacuoles. The large pericellular spaces probably contained fluid in life. × 700.

Fig. 4. Two-day explant. Haematoxylin and eosin. A tier of dilated vascular channels can be seen in the deeper subepithelial connective tissue, near the underlying muscle. × 390.

Fig. 5. Two-day explant. Sites of phosphatase activity appear black; ½ hr. incubation in substrate bath. No counterstain was employed. The stroma connective tissue cell nuclei, the epithelial cell nuclei and free borders are positive. × 280.

Fig. 6. Three-day explant. The villi are fairly uniform. Two bubble-like cysts are visible at lower right. Dilated vascular channels can be seen in the connective tissue; they were practically confluent. Haematoxylin and eosin. × 52.

Fig. 7. Three-day explant. Haematoxylin and eosin. Confluent dilated vascular channels are present. × 115.

Fig. 8. Control sample from donor used for preparation of explant illustrated in fig. 7. Haematoxylin and eosin. × 115.

Fig. 9. Three-day explant. Epithelial cells covering villus tips. Small droplet-like vacuoles can be distinguished in the infranuclear cytoplasm, especially on the tip of the right hand villus. Haematoxylin and eosin. × 700.

Fig. 10. Three-day explant. Haematoxylin and eosin. The tip of the central villus is covered by epithelium which appears to be loaded with fluid, whilst a dilated vascular channel can be seen in the connective tissue just beneath. × 350.

Fig. 11. Three-day explant. Haematoxylin and eosin. The stromal connective tissue cells are widely separated from one another as if by intercellular fluid; a dilated blood channel extends towards the villus core. Disintegrating erythrocytes could be distinguished within the latter. × 280.

Fig. 12. Control sample from donor used for preparation of explant illustrated in Fig. 13. Sites of phosphatase activity appear black. ½ hr. incubation in substrate bath. No counterstain. A positive border reaction extends continuously along the epithelial surface. × 280.

Plate 2

Fig. 13. Three-day explant, from donor illustrated in fig. 12. Sites of phosphatase activity appear black. 1 hr. incubation in substrate bath. No counterstain. A section from the periphery of an explant. The strongly positive reaction of the stromal connective tissue cells, epithelial nuclei, and free border can be seen. Large dilated spaces which probably contained fluid are clearly visible. × 700.

Fig. 14. Three-day explant, maintained in thrombin clot. 1 hr. incubation in substrate bath. Alkaline phosphatase distribution is similar to that shown in fig. 13. × 280.

Fig. 15. Control sample from donor used in preparation of explant shown in fig. 14. Sites of phosphatase activity appear black; no counterstain. ½ hr. incubation in substrate bath. The positive border reaction is clearly visible. × 280.

Fig. 16. Six-day explant. Haematoxylin and eosin. Whilst some villi are still present, the central tissue is mainly necrotic. The stromal connective tissue cells are widely separated as if the tissue had been waterlogged. × 67.

Fig. 17. Six-day explant. Sites of phosphatase activity appear black; no counterstain. ½ hr. incubation in substrate bath. The positive border reaction, epithelial nuclei and subepithelial connective tissue can be identified. Large dilated vascular channels are present. × 110.

Fig. 18. Six-day explant. Haematoxylin and eosin. An advanced degree of epithelial 'loading'. On the left, the epithelial membrane has disrupted. Compare with fig. 20. × 370.

Fig. 19. Six-day explant. Sites of phosphatase activity appear black. No counterstain. ½ hr. incubation in substrate bath. The border reaction is substantial. Positively reacting intracellular granules are present just beneath the border. The epithelial nuclei and stromal connective tissue cells are also positive. The latter are pushed apart as if by intracellular fluid. × 700.

Fig. 20. Six-day explant. Kabat and Furth's technique. ½ hr. incubation in substrate bath. An adjacent section to that illustrated in fig. 18. There is a positive reaction in the 'free border' overlying the disrupted cells. × 370.

PLATE 3

Fig. 21. Control sample from donor used to prepare explants illustrated in figs. 17 and 19. The border reaction is positive. ½ hr. incubation in substrate bath. × 280.

Fig. 22. Six-day explant. Kabat and Furth's technique. ½ hr. incubation in substrate bath. Section is from an explant which had been adjacent to that shown in fig. 19. The epithelial cells of similar appearance, yet lack a positive border reaction; their nuclei, and the subjacent connective tissue cells, however, are positive for phosphatase. × 700.

Fig. 23. Seven-day explant. Haematoxylin and eosin. The epithelial covering is fairly well preserved. Villi are present. × 150.

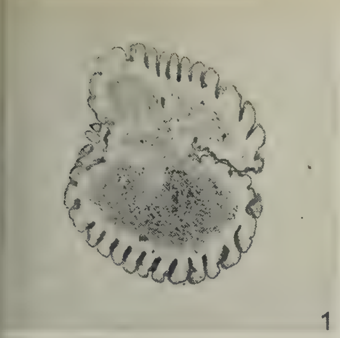
Fig. 24. Seven-day explant. Sites of phosphatase activity appear black. Section adjacent to that shown in fig. 23. 1 hr. incubation in substrate bath. The border reaction is positive in the centre of the field only. Note the small restricted positive zone lining the wall of the crypt-like structure towards the top. × 200.

Fig. 25. Seven-day explant. Sites of phosphatase activity appear black. 1 hr. incubation in substrate bath. The border reaction, though positive, is uneven, as can be seen in the intervillous spaces at the top left-hand corner and on the right. × 280.

Fig. 26. Fourteen-day explant. Haematoxylin and eosin. The villi are much reduced in height; little remains of the muscle tissue in the centre. × 42.

Fig. 27. Fourteen-day explant. Sites of phosphatase activity appear black. ½ hr. incubation in substrate bath. The border reaction is still positive but is discontinuous and patchy, as can be seen especially at the bases of the villi and in the centre. × 280.

Fig. 28. Fourteen-day explant. Bouin-fixed material. Haematoxylin and eosin. 'Loading' of the epithelial cells is clearly apparent. × 700.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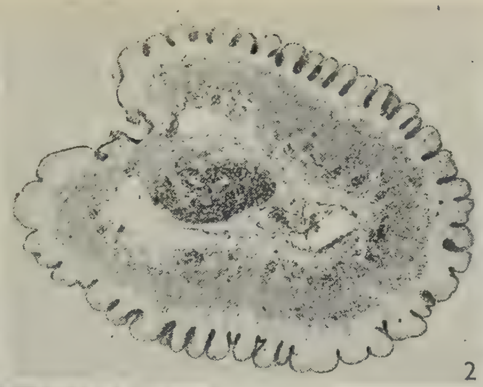
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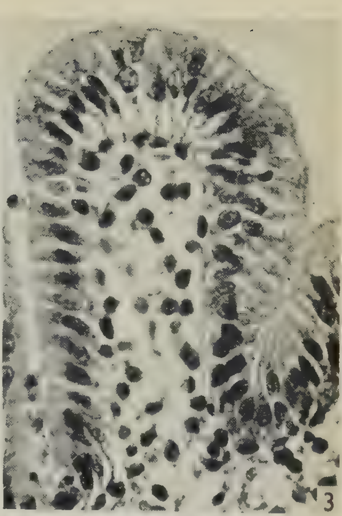
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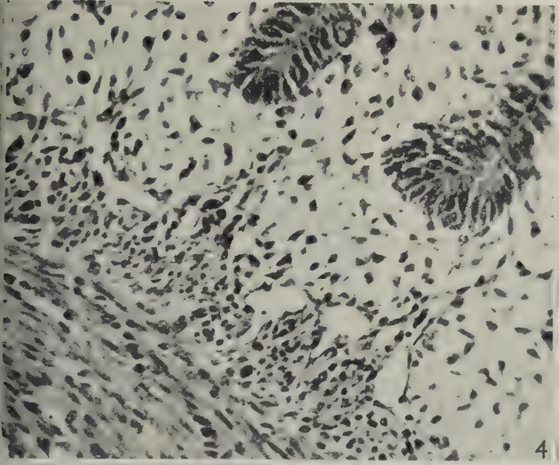
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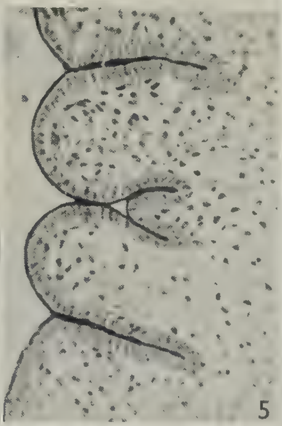
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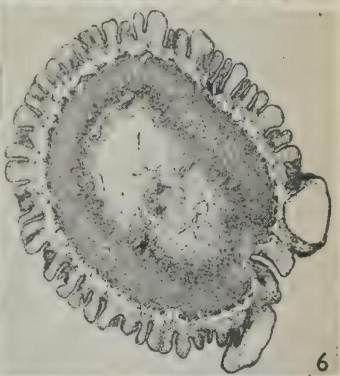
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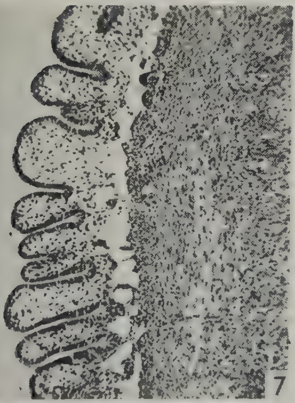
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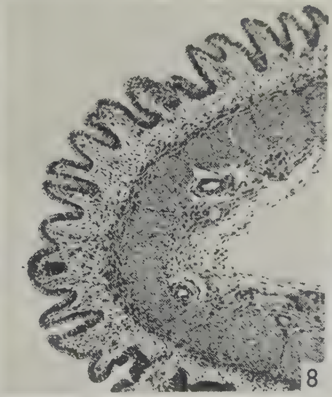
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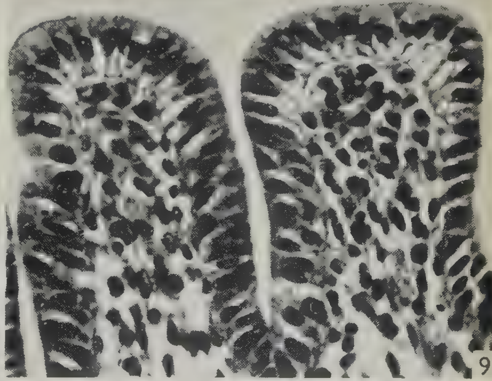
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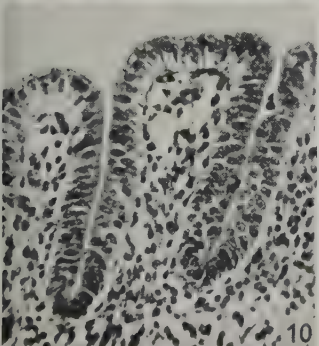
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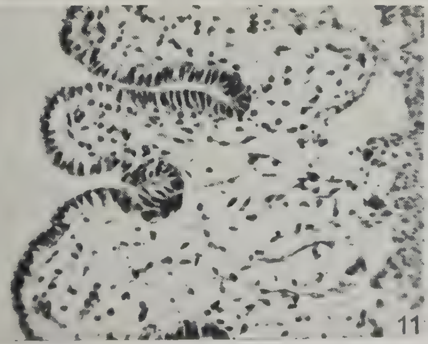
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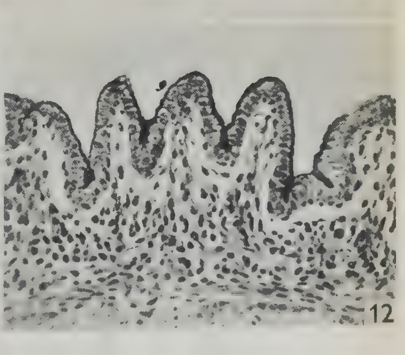
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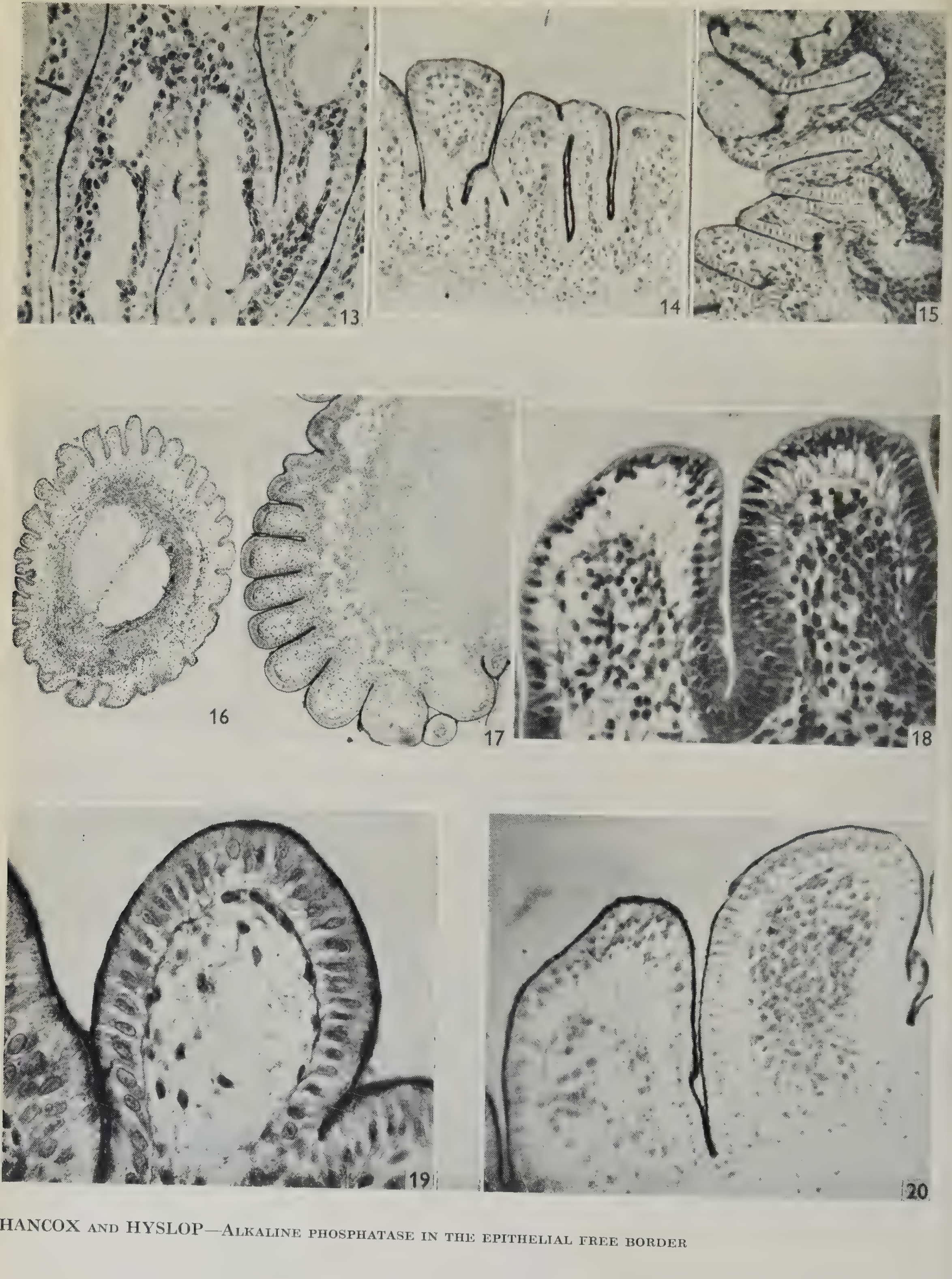
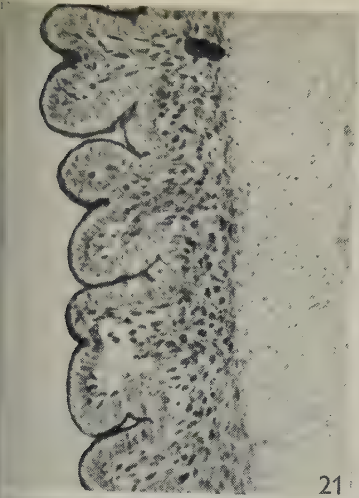


HANCOX and HYSLOP—Alkaline phosphatase in the epithelial free border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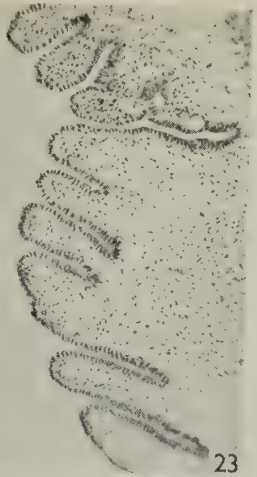
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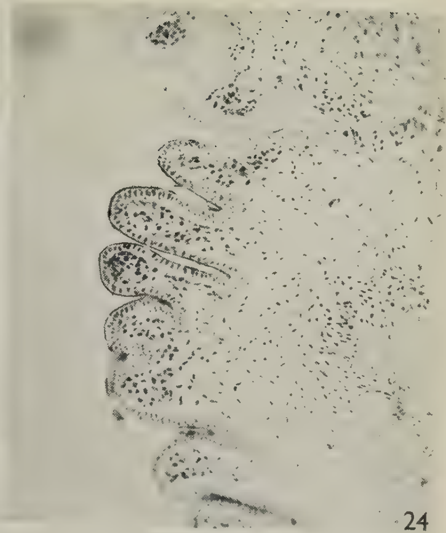
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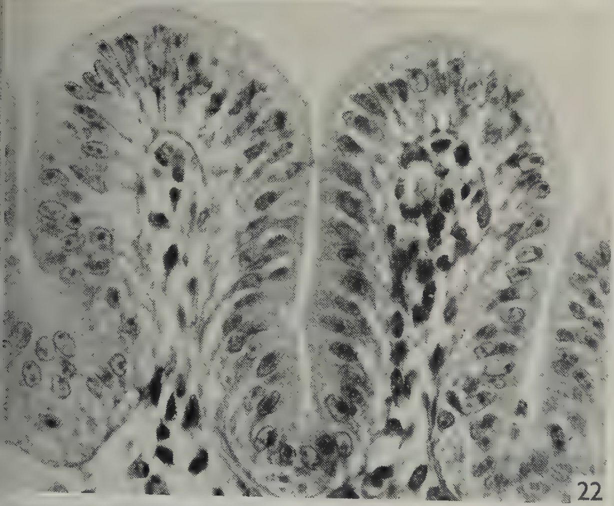
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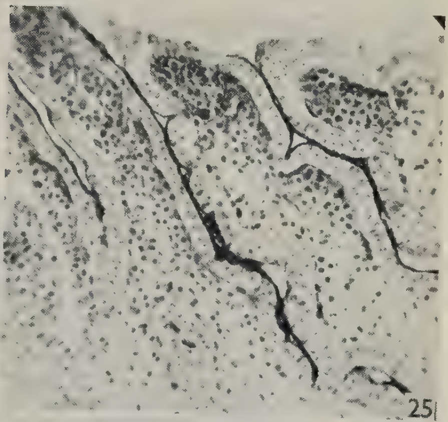
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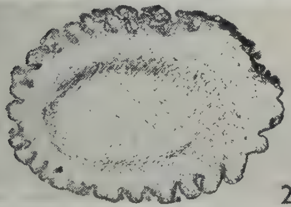
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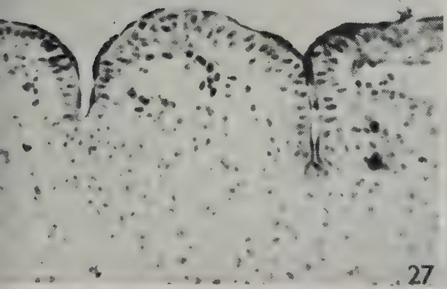
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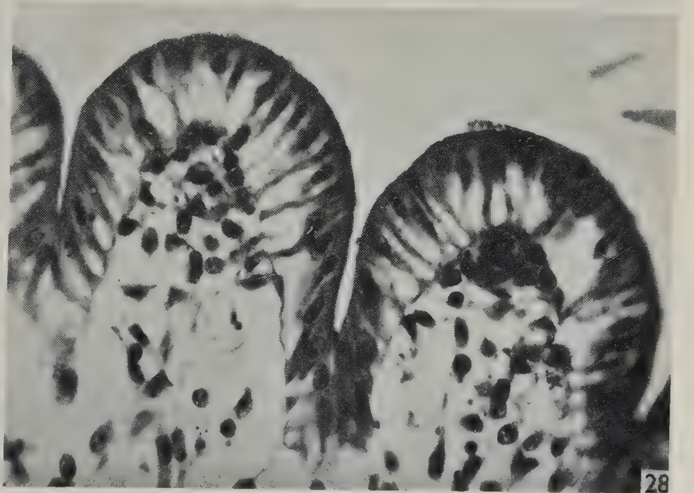
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ANCOX and HYSLOP—Alkaline phosphatase in the epithelial free border

THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE MUCOSAL CELLS OF THE SMALL INTESTINE OF THE RAT, CAT AND DOG

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INTRODUCTION

Among the many investigators who have demonstrated histochemically the presence of alkaline phosphatase in the mucosa of the small intestine, only a few have observed a bilaminar distribution of this enzyme in the superficial zones of the mucosal cells, for example, Bourne (1944) in the guinea-pig, Deane & Dempsey (1945) and Hébert (1950) in a large number of mammalian species, and Moe (1952) in the mouse and cat. Since such a distribution might have considerable physiological importance in relation to the process of absorption of some of the products of digestion, the present study was undertaken to determine whether this phenomenon is constant, and to investigate the variations in the standard technique that are necessary for its demonstration.

MATERIALS AND METHODS

The material used comprised pieces of small intestine removed from ten cats, seven rats and two dogs immediately after the animals had been killed by coal gas. From each animal nine pieces of intestine were removed, the distance of each piece from the pylorus being noted. The first piece consisted of the first centimetre of the duodenum, and the last included the terminal part of the ileum, the other pieces being taken at regular intervals from the intervening part of the small intestine. Different fixatives were used, such as varying concentrations of alcohol, formalin, formalin and sodium chloride, formalin vapour, and acetone, and it was found that fixation in 80 % alcohol for 18 hr. gave the most satisfactory results for the present purpose. In order that a strict comparison could be made between the various parts of the intestine, and between the results of variations in the techniques employed, all nine pieces from each intestine were embedded in the same paraffin block. Serial sections, 3μ thick, were cut and mounted in distilled water, instead of the usual glycerine-albumen mounting medium, as the latter was found to give marked variations in the intensity of the reaction from one part to another of the same histological section. The method used for revealing the site of the alkaline phosphatase was the modification by Kabat & Furth (1941) of the Gomori (1939) technique, in which Mg ions are added as an enzyme activator. The sections were incubated in a substrate solution containing sodium-β-glycerophosphate at a constant pH of 9·4 for periods varying between 5 min. and 24 hr., after which they were placed in a 2 % solution of cobalt nitrate for 5 min. A few sections were left in the cobalt nitrate for times up to 30 min. to test if a diffusion of cobalt phosphate might occur during the carrying out of this part of the technique.

During the final step of the technique the sections were placed in solutions of ammonium sulphide of varying strengths and 'maturity' for periods ranging from 2 to 50 min. It was found that this step in the technique was the critical one for the demonstration of a bilaminar reaction at the free surface of the mucosal cells of the intestine. Gomori (1939), in his original description, suggested that the ammonium sulphide should be dark yellow in colour and that a few drops should be added to a Coplin jar of distilled water. In the present work three different ammonium sulphide solutions were used. Solution 'A' consisted of freshly prepared ammonium sulphide which was lemon in colour and smelt strongly of hydrogen sulphide; solution 'B' consisted of ammonium sulphide which had been allowed to age until it had become deep yellow and smelt of ammonia; and solution 'T' was prepared by adding sodium iodate to freshly prepared ammonium sulphide, a procedure which artificially ages the solution in a few hours. It is probable that the solution 'B' described above corresponds to the ammonium sulphide used by Gomori. Each solution was tested at strengths ranging between 0·04 and 1 % for varying times.

RESULTS

Throughout this work care has been taken to ensure that differing results in each of the various trials could have been produced by a difference in only one step of the technique. As far as possible, when variations in any one step were being studied, all the sections, including controls, were taken through the other steps of the procedure at the same time.

A. *Variations in the last step in which the sections are placed in the ammonium sulphide solution*

It was found that irrespective of the particular ammonium sulphide used three different types of reaction could be produced at the free surfaces of the mucosal cells of the small intestine. The first reaction (Pl. 1, fig. 1), consisted of two dark bands, one of which was situated at the free surface of the striated border and the other in the region of the apical cytoplasm. Between these two bands there was a clear, non-staining zone. The second reaction (Pl. 1, fig. 2), which occurred when the period during which the sections were placed in the ammonium sulphide solution was increased, differed from the first in that the clear zone between the two bands had partly disappeared. On further increasing the length of this final step of the technique the third reaction appeared (Pl. 1, fig. 3). This differed from the previous two reactions in that the two dark bands became blended into one broad band and the clear zone between them completely disappeared. The length of time which was required to produce these reactions varied with the ammonium sulphide used and with its strength (see Table 1). Decreasing the age and increasing the strength of the ammonium sulphide solution increased the rate at which any reaction occurred.

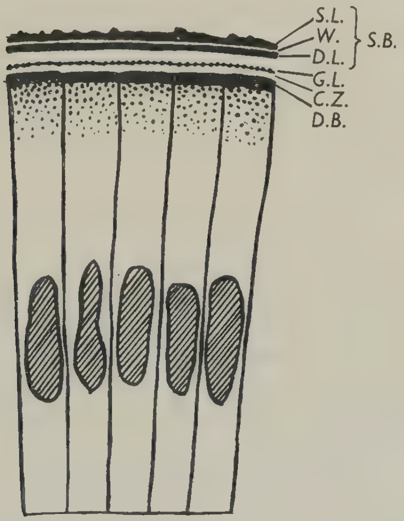
The exact localization of these bands becomes more evident when the section is examined under high magnification (Pl. 3, fig. 16), and when it is compared with a similar section stained with haematoxylin and eosin and magnified to the same extent (Pl. 3, fig. 17). It will be noted that at the higher magnification a third band

(*G.L.* in Pl. 3, fig. 16, and Text-fig. 1), intermediate in position, can be recognized, that appears to correspond in position to the granular layer (Baker, 1944) which can be plainly seen in the section stained with haematoxylin and eosin (*G.L.* in Pl. 3, fig. 17). The superficial band, which is situated at the free edge of the striated border, shows on close examination, a thin pale line (*W.* in Pl. 3, fig. 16, and Text-fig. 1), dividing it into two longitudinal layers, the superficial of which (*S.L.* in Pl. 3, fig. 16, and Text-fig. 1) appears to be loosely applied to the border, whilst the deeper layer (*D.L.* in Pl. 3, fig. 16, and Text-fig. 1) seems to be part of the free edge of the border. The deepest band (*D.B.* in Pl. 3, fig. 16, and Text-fig. 1) appears to be situated in that portion of the cytoplasm lying below the granular layer, and the clear zone immediately external to it represents the thin clear zone of cytoplasm (*C.Z.* in Pl. 3, figs. 16, 17, and Text-fig. 1), lying deep to the granular layer, these sites being determined by comparison with the haematoxylin and eosin section.

Table 1. *The effect of using three different ammonium sulphide solutions, for varying times, on the appearance of the striated border of the small intestine of the cat*

(The incubation time in the substrate in all cases was 60 min. at 37° C. All times are in minutes.)

Ammonium sulphide	Strength (%)	Appearance at various times Double layer	 Double layer closing up	 Single layer
A	0·04	7	25	45
	0·1	2	6	10
B	0·2	8	30	45
	0·5	2	4	6
T	0·4	12	30	50
	1·0	5	9	12



Text-fig. 1. Diagrammatic representation of the localization of alkaline phosphatase at the free surfaces of the mucosal cells of the small intestine.

In the opinion of the authors, the third or intermediate band (*G.L.* in Pl. 3, fig.16) does not represent a true area of phosphatase activity but is produced by the opacity of the granular layer at the base of the striated border.

B. *Variations in the period of incubation*

During this stage of the work the sections were incubated for differing times, and during the final step of the technique they were all placed in the same ammonium sulphide solution for the same time, it having been determined beforehand which ammonium sulphide solution at a given strength, and used for a given time, gave a bilaminar reaction in the region of the striated border.

When the sections were incubated for 5 min. in the substrate solution a definite bilaminar reaction was seen at the free surfaces of the intestinal cells (Pl. 1, fig. 6). The cytoplasm and nuclei were faintly positive but the Golgi zone appeared negative. With an incubation period of 25 min. there was still the same bilaminar reaction at the free surfaces of the cells, whilst the cytoplasm and nuclei were slightly more positive than at 5 min., and in some cells there was a faint reaction in the Golgi zone (Pl. 1, fig. 7). Incubation for 60 min. caused an increase in the reaction of the cytoplasm, especially in the region of the Golgi apparatus. With this period of incubation the nuclei of the mucosal cells gave a strongly positive reaction and some of the nuclei of the submucosa became positive (Pl. 1, fig. 8). At the end of 120 min. incubation there was a further general increase in the intensity of reaction, especially in the nuclei of the cells of the submucosa. The bilaminar reaction appeared the same as with the shorter periods of incubation (Pl. 1, fig. 9). It was noted that the bilaminar reaction was the same, no matter how long the period of incubation, and even periods up to 24 hr. produced only a general increase in the intensity of the reaction.

C. *Variations in sectioning and mounting*

For the demonstration of the site of alkaline phosphatase activity in the intestine the authors have found that sections cut at 3μ give a more easily interpreted picture than do sections of greater thickness. The thickness of the section is of prime importance when the exact localization in the striated border is being studied, as owing to the cylindrical nature of the villi thick sections tend to obscure the bilaminar reaction due to the overlapping of the various bands. Even with thin sections it was quite often found that the tips of the villi were cut obliquely and that the bilaminar reaction present in the sides of the villi appeared to become a single band at the apices (Pl. 2, fig. 15).

In the early stages of this work many sections were found to show marked variations in the intensity of reaction between one area and another (Pl. 2, fig. 14). This was found to be due to the use of a glycerine-albumen mounting medium, the glycerine of which inactivated the enzyme during the drying of the sections. Subsequently, mounting with distilled water was carried out, a process which gave a uniform intensity of the reaction throughout the section.

D. *Variations in the reaction in different parts of the small intestine, using a constant technique*

The pieces of intestine from each animal were treated by the technique which had been shown, in an earlier stage of the work, to give a constant demonstration of the bilaminar reaction at the free surfaces of the mucosal cells.

Dog. Throughout the length of the small intestine the cytoplasm of the mucosal cells gave a positive reaction. The cytoplasm between the nuclei and the free surface of the cells (superficial cytoplasm) gave a more dense reaction than that deep to the nuclei. The reaction was also noted to be stronger in the cells near the apices of the villi than in those nearer their bases. When different segments were compared it was found that there was a gradual decrease in intensity along the length of the intestine from the pylorus to the ileo-caecal valve.

The striated border was positive over the entire height of the villi, except in the last tenth (15 cm.) of the intestine where the striated border of the cells at the bases of the villi was negative. In the proximal half of the intestine the reaction in the striated border was bilaminar (Pl. 1, fig. 4) throughout the whole height of the villi. The extent of distribution of this bilaminar reaction in the villi gradually decreased in succeeding portions of the intestine, and in the terminal ileum it was present only at their apices. Where the bilaminar reaction was decreased in extent in the villi the striated border in other parts of these villi showed a positive reaction in the form of a single band (Pl. 1, fig. 5), except in the terminal ileum where the border was negative at the bases of the villi.

Cat. As in the dog, the cytoplasm of the mucosal cells of the cat's intestine was more strongly positive in the superficial regions of the cells (Pl. 1, fig. 7) and at the tips of the villi; it also showed a gradually decreasing intensity of reaction from the duodenum to the terminal ileum.

The striated border was positive throughout the villi only in the proximal half of the intestine; the distal half (Pl. 2, fig. 12) showed increasing negative areas at the bases of the villi until in the terminal ileum the striated border on the basal third of each villus was negative. The ratio of the extent of a bilaminar reaction to a single-line reaction varied along the length of the intestine. In the first part of the duodenum only the striated border on the apices of the villi showed the bilaminar reaction; this was followed by a length of intestine, approximately half of the total, in which the reaction was bilaminar for two-thirds of the depth of the villi; then followed a portion in which the extent of the bilaminar reaction gradually decreased until in the terminal third of the intestine the reaction consisted of a single band over the whole extent of the villi.

Rat. Although the technique used in investigating the localization of alkaline phosphatase in the small intestine of the rat was exactly the same as that used for the dog and cat, the reaction in those areas which were positive was much stronger than in the other two animals (Pl. 2, fig. 10). This was particularly noteworthy in the cytoplasm, which in the proximal third of the intestine gave a dense reaction with no variations between the cells at the tips and bases of the villi. The reaction of the cytoplasm of the middle third of the intestine was similar to that of the dog and cat in that the superficial portions 'stained' more densely than the basal portions and the cells at the apices of the villi were more strongly positive than those at their bases (Pl. 2, fig. 13). In the terminal third of the intestine there was a rapid falling off of the intensity of the reaction of the cytoplasm, and the cytoplasm of the cells in the lower 10 cm. or so of the ileum gave a negative reaction.

The reaction of the striated border was positive throughout the villi in the upper half of the intestine, but in the lower half less and less of the border was positive, only small scattered areas being positive at the tips of the villi in the distal third of the intestine (Pl. 2, fig. 11). The reaction was bilaminar in form throughout the greater part of the intestine, except at the lower end where the small positive areas at the tips of the villi consisted of a single band.

DISCUSSION

Although a few investigators have noted the presence of a bilaminar reaction at the cuticular surface of the mucosal cells of the intestine, no detailed study has been made of the distribution of this reaction or of the factors which may be involved in its demonstration. Bourne (1944) and Deane & Dempsey (1945) merely mention the presence of a bilaminar reaction. Hébert (1950) and Moe (1952) both considered it to be produced by diffusion during prolonged periods of incubation.

The present work shows that, during the final step of the Gomori technique, in which the sections are placed in ammonium sulphide, three different reactions can be produced at the free surfaces of the mucosal cells of the intestine. There appears to be two possible explanations of this variation in the histological picture. First, the positive bands may represent regions with a high concentration of enzyme and the clear zones between them might be enzymically inactive regions. If this be so, then the disappearance of the clear zone, when the period in which the sections are placed in ammonium sulphide is increased, may indicate a diffusion of some product of reaction during the carrying out of the technique. Martin & Jacoby (1949), Gomori (1950, 1951), Yokoyama, Stowell & Mathews (1951), Novikoff (1951) and Moog (1951) have shown that a process of diffusion does occur during the incubation of sections in the substrate, and, whilst there seems to be some doubt as to whether it is the enzyme or the calcium phosphate that diffuses, most investigators consider that the latter substance is the diffusing agent. This problem of diffusion has thrown some doubt on the validity of the Gomori technique for the demonstration of the exact localization of alkaline phosphatase; however, when short periods of incubation are used, it does seem to be much more sensitive in demonstrating sites of low enzymic concentration than the azo-dye method of Manheimer & Seligman (1948).

In this investigation the appearances suggest that diffusion occurs into the submucosal cells of the intestine and possibly to the nuclei of the mucosal cells when the period of incubation is increased beyond 25 min. However, with incubation periods up to 24 hr. the bilaminar reaction always remains constant, a fact which appears to indicate that the replacement of the two bands by a single band is not due to diffusion of either the enzyme itself or the product of incubation, namely, calcium phosphate. Neither did prolonged periods in the cobalt nitrate have any influence on the appearance of the bilaminar reaction. Thus, as variations in the technique before the final step do not affect the bilaminar reaction, any change from a bilaminar to a single reaction, if due to diffusion, must be produced by a diffusion of the cobalt sulphide. This, however, is unlikely since there is no evidence of diffusion of the cobalt sulphide into the subjacent cytoplasm and nuclei of the epithelial cells during prolongation of this step of the technique (Pl. 1, fig. 6).

The second explanation may be that there are areas of high and low enzymic concentration in the free surfaces of the cell. If this were so it would be expected that the reaction between the cobalt phosphate and the ammonium sulphide would occur more quickly and give a more intense reaction in those regions with the highest concentration. Those regions with low enzymic activity, that is the clear zones in the neighbourhood of the striated border, would need a longer time in the ammonium sulphide to give a positive reaction, since the amount of cobalt phosphate formed would be proportionate to the amount of enzyme present.

No matter which of these explanations is accepted the findings of this work are highly suggestive of a distribution of alkaline phosphatase in a bilaminar manner at the free surfaces of the mucosal cells.

In the intestines of the three species examined there appears to be a definite difference in the distribution of alkaline phosphatase in the mucosal cells. There is a difference not only between one species and another, but also between different parts of the intestine of one and the same animal. Along its length, each intestine shows a gradient of intensity of 'staining' and also a gradient of distribution of the bilaminar reaction at the striated border, the gradient of the bilaminar reaction falling more rapidly than the gradient of intensity.

In the cat and dog both gradients show a gradual decline along the length of the intestine from the duodenum to the terminal ileum, in contrast to which the duodenum and upper jejunum of the rat show relatively constant reactions which are followed by steep gradients in the terminal jejunum and upper ileum, the last 20 cm. of the ileum being completely negative.

There has been much speculation as to the functional significance of alkaline phosphatase in the small intestine. Cori (1925) showed that the larger molecules of the hexoses (galactose and glucose) were more rapidly absorbed from the intestine than the smaller molecules of the pentoses (xylose and arabinose), a fact which suggested an active absorption of the former sugars. Bárány & Sperber (1939) gave definite proof of the active absorption of glucose by demonstrating that it is capable of being absorbed against a concentration gradient existing between the intestinal contents and the portal blood. To explain the different rates of absorption of sugars Lundsgaard (1933) and Verzár and his co-workers (Verzár & McDougall, 1936) postulated that during the absorption of those sugars which are rapidly absorbed a phosphorylation process occurs. In support of this theory of phosphorylation Verzár showed that the rate of absorption of glucose and galactose is independent of their concentration, and that these two sugars undergo phosphorylation *in vitro* and *in vivo*. On the other hand, the pentoses, xylose and arabinose, do not undergo phosphorylation, and their rates of absorption are dependent on their concentration in the intestine. Verzár further showed that when animals are treated with the metabolic poisons, monoiodoacetic acid and phloridzin, the absorption of glucose and galactose is markedly decreased whilst the absorption of arabinose and xylose remains constant. He believed that monoiodoacetic acid and phloridzin inhibited the phosphorylation of glucose and galactose, thus abolishing their selective absorption.

On theoretical grounds, phosphorylation should be capable of increasing the rate of glucose absorption by the formation of concentration gradients across the intestinal epithelium. Glucose will diffuse into the intestinal cells as long as its concentration in the lumen of the intestine is higher than that in the interior of the cell. Should the glucose accumulate in the interior of the cell then its concentration would soon equal that in the intestinal lumen and further diffusion into the cell would cease. However, if glucose, on entering the cell, is rapidly phosphorylated or esterified to form glucose phosphate the concentration gradient of glucose across the superficial membrane of the cell will be maintained and diffusion will continue. Since glucose exists only in its free form in the portal blood the glucose phosphate formed in the superficial part of the cell must undergo hydrolysis at some deeper part of the cell before passing into the blood stream. This hydrolysis or dephosphorylation of glucose phosphate will of itself cause a concentration gradient of glucose phosphate in the interior of the cell and also cause a sufficiently high concentration of glucose in the cell to produce a diffusion of this latter substance across the inner cell membrane into the portal blood. This system of esterification and hydrolysis would necessitate the presence of phosphatase in two layers in the mucosal cells. One of the chief objections to this theory of phosphorylation as the mechanism of active absorption of glucose in the intestine has been that, although phosphatase is capable of causing rapid hydrolysis, it is incapable of synthesizing biological phosphate esters which are essential for the formation of concentration gradients. Recently, however, Meyerhof & Green (1950) have shown that in the presence of high-energy phosphate donors, which are present in practically all tissues of the body, transphosphorylation by alkaline phosphatase can occur.

If some of the products of digestion are phosphorylated during absorption, then it might be postulated that the two bands of phosphatase which have been described would be capable of carrying out the processes of esterification and hydrolysis at the luminal surfaces of the mucosal cells.

It is unfortunate that in the literature there do not seem to be comparable figures for the rates of absorption of sugars from the intestines of the three animals which have been studied. However, Davidson & Garry (1940) found that glucose is not so rapidly absorbed from the intestine of the cat as from that of the rat, and Fisher & Parsons (1950) have shown that in the rat, even when the gradient of mucosal surface area in the small intestine and the gradient of utilization of glucose by the tissues of the intestinal wall are taken into account, there is a gradient of 'translocation' of glucose across the mucosa. These two findings would seem to favour the theory of phosphorylation when they are considered in the light of the present observations that the upper portion of the rat's intestine gives a much more positive reaction for alkaline phosphatase than the cat's, and that in the rat's intestine there is a gradient of intensity of reaction and also a gradient of distribution of the bilaminar reaction.

Ancillary evidence, derived from a study of the distribution of alkaline phosphatase in the kidney, of the localization of this enzyme in a bilaminar manner in the proximal convoluted tubules, where glucose is actively absorbed, will be presented in another paper by one of the present authors (Johnson).

SUMMARY

1. The presence of a bilaminar reaction at the free surfaces of the mucosal cells of the intestine has been confirmed.

2. This reaction can be constantly demonstrated by a modification of the Gomori technique which is described.

3. The effect of varying the period of incubation has been studied from the point of view of the bilaminar reaction and the phenomenon of diffusion.
4. In the small intestine of the rat, cat and dog a gradient of intensity of the general reaction and a gradient of bilaminar reaction have been observed.
5. The observations made in this work are discussed in relation to the process of phosphorylation.

The authors wish to express their sincere thanks to Prof. Francis Davies and Prof. H. A. Krebs for helpful criticisms and suggestions. Part of the expense of this work was borne by a grant which one of us (F. R. J.) has received from the Sheffield University Medical Research Fund.

REFERENCES

Baker, J. R. (1944). The free border of the intestinal epithelial cell of vertebrates. *Quart. J. micr. Sci.* **84**, 73–105.

Bárány, E. & Sperber, E. (1939). Absorption of glucose against a concentration gradient by the small intestine. *Skand. Arch. Physiol.* **81**, 290–299.

Bourne, G. (1944). The distribution of alkaline phosphatase in various tissues. *Quart. J. exp. Physiol.* **32**, 1–20.

Cori, C. F. (1925). The fate of sugar in the animal body. I. The rate of absorption of hexoses and pentoses from the intestinal tract. *J. biol. Chem.* **66**, 691–715.

Davidson, J. N. & Garry, R. C. (1940). The absorption of monosaccharides from the distal small intestine of anaesthetized cats. *J. Physiol.* **97**, 509–516.

Deane, Helen W. & Dempsey, E. W. (1945). The localisation of phosphatases in the Golgi region of intestinal and other epithelial cells. *Anat. Rec.* **93**, 401–417.

Fisher, R. B. & Parsons, D. S. (1950). Glucose absorption from surviving rat small intestine. *J. Physiol.* **110**, 281–293.

Gomori, G. (1939). Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. exp. Biol., N.Y.*, **42**, 23–26.

Gomori, G. (1950). Sources of error in enzymatic histochemistry. *J. Lab. clin. Med.* **35**, 802–809.

Gomori, G. (1951). Alkaline phosphatase of cell nuclei. *J. Lab. clin. Med.* **37**, 526–531.

Hébert, Simone (1950). Les phosphatases alcalines de l'intestin Étude histochemique expérimentale. *Arch. Biol., Paris*, **61**, 235–289.

Kabat, E. A. & Furth, J. (1941). A histochemical study of the distribution of alkaline phosphatase in various normal and neoplastic tissues. *Amer. J. Path.* **17**, 303–318.

Lunsgaard, E. (1933). Hemmung von Esterifizierungsvorgängen als Ursache Phlorrhizinwirkung. *Biochem. Z.* **264**, 209–220.

Manheimer, L. H. & Seligman, A. M. (1948). Improvement in the method for the histochemical demonstration of alkaline phosphatase and its use in a study of normal and neoplastic tissues. *J. Nat. Cancer Inst.* **9**, 181–199.

Martin, B. F. & Jacoby, F. (1949). Diffusion phenomenon complicating the histochemical reaction for alkaline phosphatase. *J. Anat., Lond.*, **83**, 351–363.

Meyerhof, O. & Green, H. (1950). Synthetic action of phosphatase. II. Transphosphorylation by alkaline phosphatase in the absence of nucleotides. *J. biol. Chem.* **183**, 377–390.

Moe, H. (1952). On the histochemical demonstration of alkaline glycerophosphatase and its dependence on the incubation time. *Anat. Rec.* **112**, 217–235.

Moog, Florence (1951). Non-diffusibility of alkaline phosphatase in fixed tissue. *Science*, **114**, 109.

Novikoff, A. B. (1951). The validity of histochemical phosphatase methods on the intracellular level. *Science*, **113**, 320–325.

Verzár, F. & McDougall, E. J. (1936). *Absorption from the Intestine*, pp. 113–149. London: Longmans, Green and Co.

Yokoyama, H. O., Stowell, R. E. & Mathews, R. M. (1951). Evaluation of histochemical alkaline phosphatase technics. *Anat. Rec.* **109**, 139–160.

EXPLANATION OF PLATES

All the photomicrographs, except fig. 17, are of sections demonstrating the presence of alkaline phosphatase. No counterstaining was used.

Plate 1

Fig. 1. Jejunum of cat. Incubation time 60 min. Bilaminar reaction in the striated border, positive Golgi zone and positive nuclei. × 530.

Fig. 2. Jejunum of cat. Incubation time 60 min. Bilaminar reaction closing. × 530.

Fig. 3. Jejunum of cat. Incubation time 60 min. Reaction at the striated border in the form of a single layer. × 530.

Fig. 4. Jejunum of dog. Incubation time 5 min. Bilaminar reaction in the striated border, faintly positive superficial cytoplasm. × 530.

Fig. 5. Ileum of dog. Incubation time 5 min. Single reaction in the striated border at the base of a villus. × 530.

Fig. 6. Jejunum of cat. Incubation time 5 min. Bilaminar reaction, faintly positive superficial cytoplasm. × 530.

Fig. 7. Jejunum of cat. Incubation time 25 min. Bilaminar reaction. Cytoplasm, nuclei and Golgi zone faintly positive. × 530.

Fig. 8. Jejunum of cat. Incubation time 60 min. Bilaminar reaction. Positive cytoplasm, Golgi zone, and mucosal and submucosal nuclei. × 530.

Fig. 9. Jejunum of cat. Incubation time 120 min. Bilaminar reaction. Strongly positive cytoplasm, Golgi zone, and mucosal and submucosal nuclei. × 530.

Plate 2

Fig. 10. Duodenum of rat. Incubation time 20 min. Bilaminar reaction in the striated border; entire cytoplasm of the mucosal cells strongly positive. × 530.

Fig. 11. Lower ileum of rat. Incubation time 20 min. Scattered positive areas in the striated border at the tips of the villi. Submucosal cells positive. × 130.

Fig. 12. Ileum of cat. Incubation time 20 min. Negative reaction in the striated border at the bases of the villi, with single layer on their sides and bilaminar reaction towards their apices. × 130.

Fig. 13. Jejunum of rat. Incubation time 20 min. Bilaminar reaction in the striated border; superficial cytoplasm and Golgi zone positive. × 530.

Fig. 14. Jejunum of cat. Incubation time 25 min. Showing irregular reactions due to the action of glycerine-albumen mounting medium. × 40.

Fig. 15. Jejunum of cat. Incubation time 60 min. Showing the change from a bilaminar reaction on the side of the villus to a single reaction at the tip, the appearance being due to obliquity of the section. × 530.

Plate 3

Fig. 16. Jejunum of cat. Incubation time 5 min. Bilaminar reaction with intermediate third (granular) layer. × 2600.

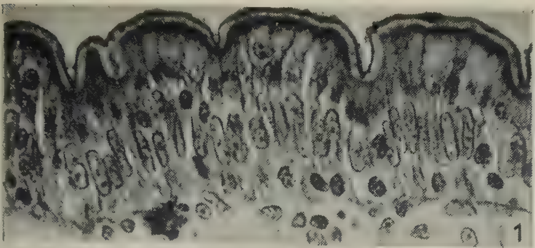
Fig. 17. Jejunum of cat. Stained with haematoxylin and eosin showing the granular layer at the base of the striated border. × 2600.

LIST OF ABBREVIATIONS

C.Z.	Clear zone.
D.B.	Deep band.
D.L.	Deep layer of superficial band.
G.L.	Granular layer.
S.B.	Superficial band.
S.L.	Superficial layer of superficial band.
W.	Pale line dividing superficial and deep layers of superficial band.

ADDENDUM

Since this paper was written, Professor J. F. Danielli has kindly provided the authors with pieces of small intestine of the cat which had been freeze-dried. Sections of this material prepared and treated by the methods described in the present paper also revealed a bilaminar reaction in the mucosal cells.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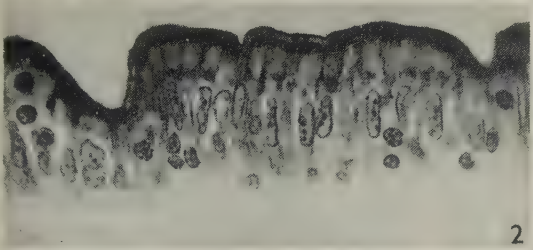
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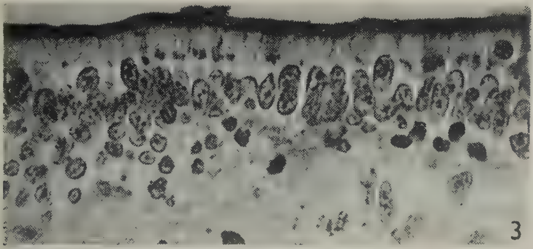
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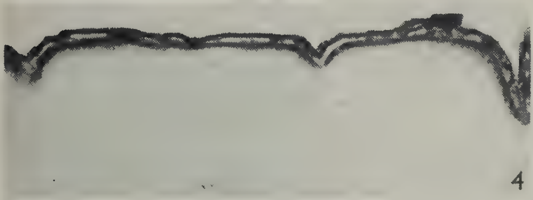
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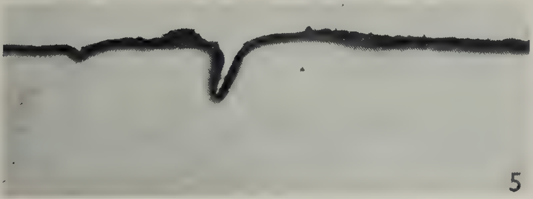
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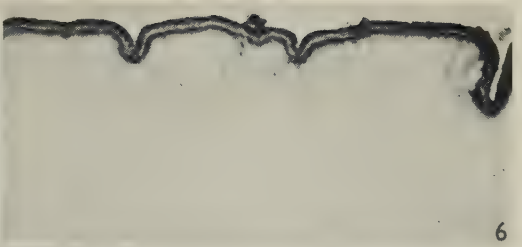
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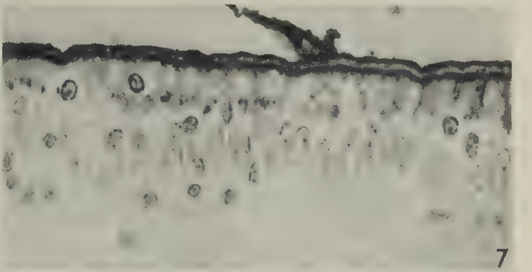
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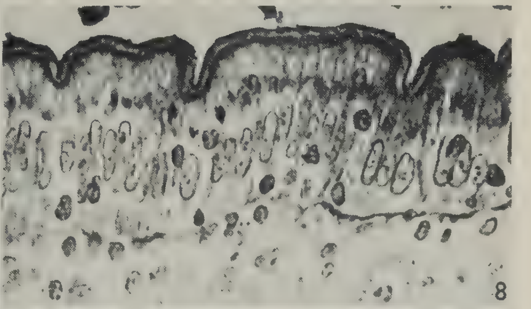
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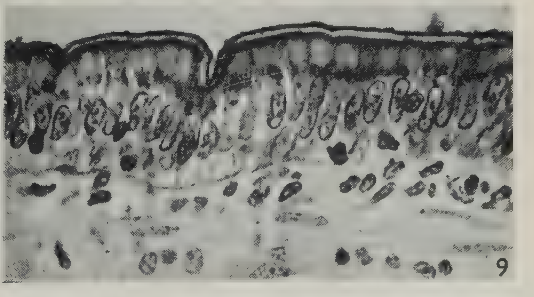
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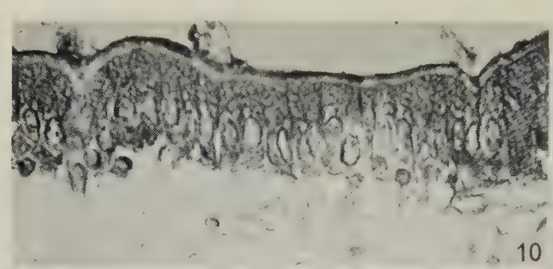
JOHNSON AND KUGLER—ALKALINE PHOSPHATASE IN THE MUCOSAL CELLS OF SMALL INTESTINE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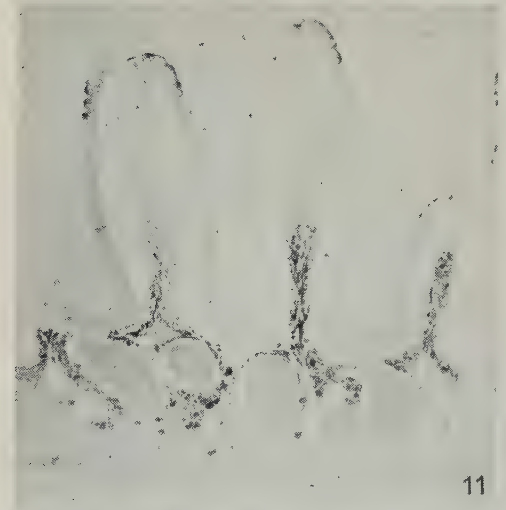
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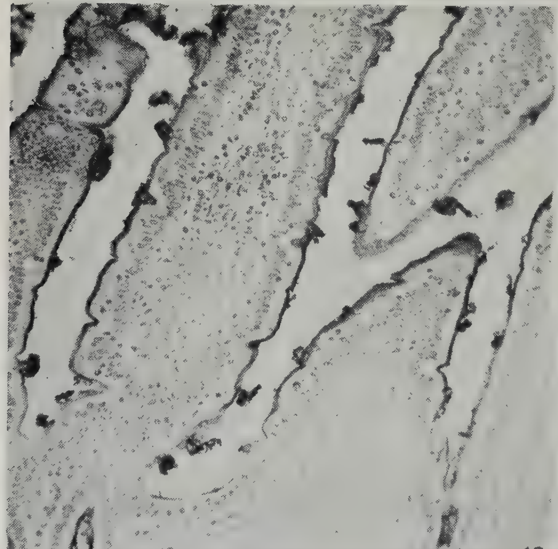
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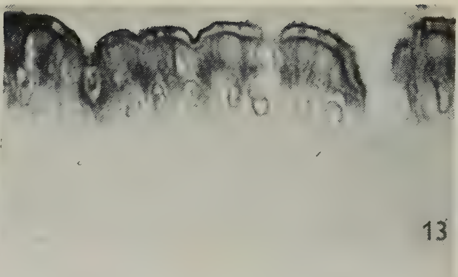
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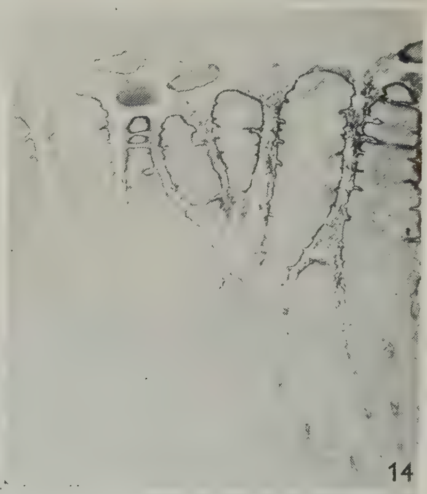
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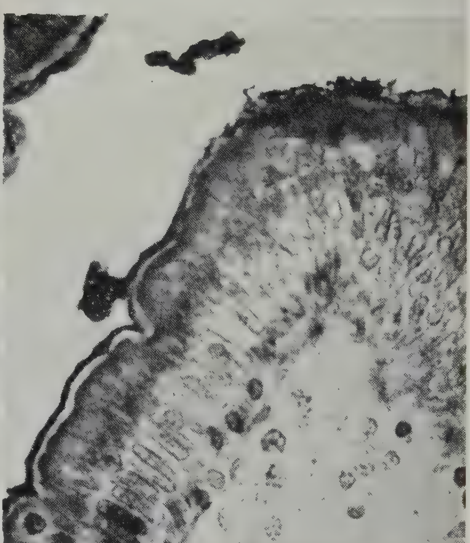
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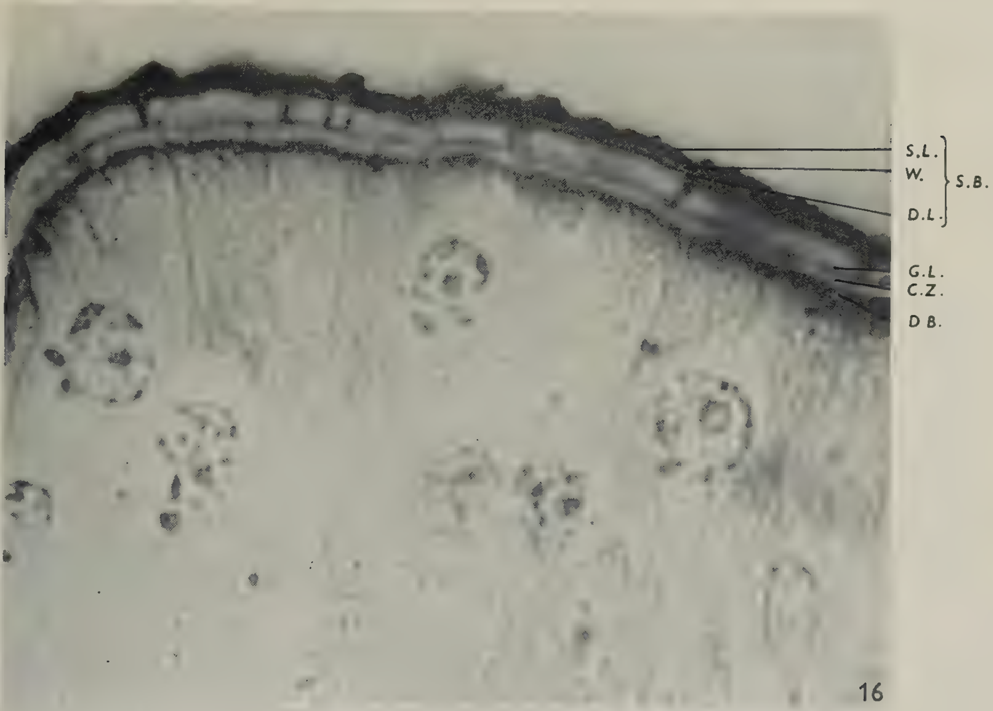
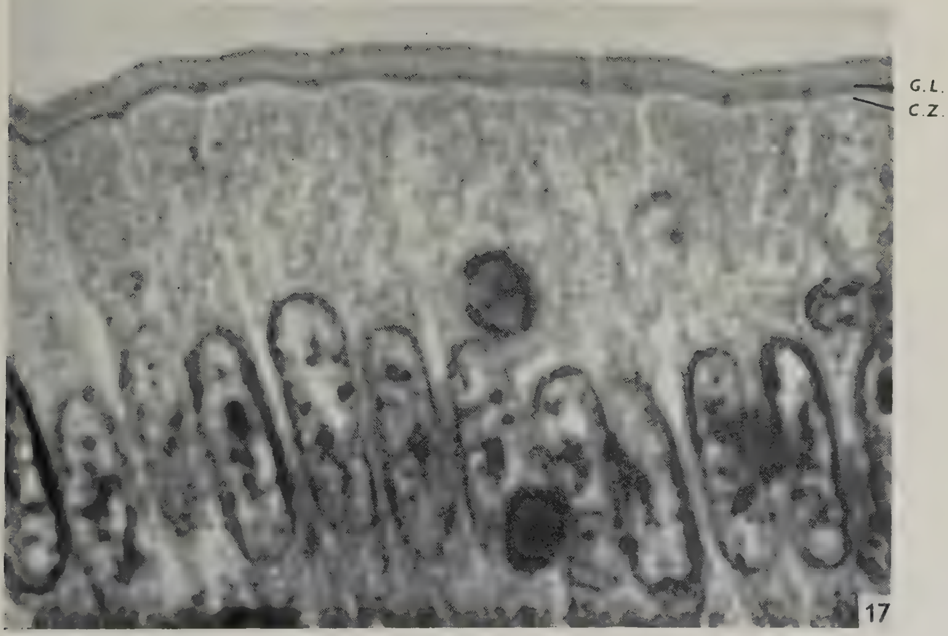
JOHNSON AND KUGLER—ALKALINE PHOSPHATASE IN THE MUCOSAL CELLS OF SMALL INTESTINE





JOHNSON AND KUGLER—ALKALINE PHOSPHATASE IN THE MUCOSAL CELLS OF SMALL INTESTINE

THE VASCULARIZATION OF THE RAT PANCREAS AND THE EFFECT OF ISCHAEMIA ON THE ISLETS OF LANGERHANS

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Department of Anatomy, University of Liverpool

The pancreas develops as diverticula from the embryonic alimentary canal at the junction of the fore- and mid-guts, and derives its arterial supply from branches of both the coeliac and superior mesenteric arteries. An investigation of the extent of vascularization of the pancreas from each of these sources by means of arteriography and planned interruption of pancreatic vessels, with a view to correlating this with the development of the pancreas, would be of great interest. The anastomoses between pancreatic vessels, however, are most efficient and consequently it was decided that a study of the details of these anastomoses and of the histological and physiological responses of the pancreas to ischaemia, with particular reference to the development of adequate collateral arterial channels, would yield information of some value.

MATERIAL AND METHODS

Albino rats, fed on a uniform diet of rat cake (Lever's Cattle Foods, Ltd., Bebington Cheshire), were used for the investigation.

The normal arterial pattern was determined in thirty rats and the venous drainage in five. Immediately after killing each rat by an overdose of chloroform, a cannula was introduced into the thoracic aorta, and either 20% bismuth oxychloride suspension or 10% 'Chlorbismol' (May and Baker) injected. The venous drainage was examined after injecting a rice starch-gelatine mixture through the portal vein, by Scharrer's (1940) method.

The vessels were observed *in situ* and then after removal of the stomach, duodenum, transverse colon and spleen together with the pancreas. The application of the Spalteholtz technique to the exsected viscera was carried out in the majority of animals. Radiographic examination, using a fine grain film (Kodaline) has been a valuable aid to elucidating the vascular pattern.

As a method of investigating the degree of anastomosis between pancreatic arteries, and determining the functional value of individual vessels, planned interruption of these arteries was carried out. One hundred and fifteen animals were used for this study. The splenic artery was interrupted at its origin in fifty-one rats and the pancreas and its vessels examined after periods of 1 day to 112 days. In fourteen animals the superior pancreatico-duodenal, the inferior pancreatico-duodenal and the splenic arteries were interrupted, and the pancreas examined after intervals of 3–21 days. The splenic, gastro-duodenal, inferior pancreatico-duodenal and inferior pancreatic arteries were divided in twenty-two rats, and the effect observed after intervals of 1–21 days.

The remaining animals were subjected to interruption of the splenic and superior pancreatico-duodenal arteries (two rats); the splenic and inferior pancreatico-duodenal arteries (three rats); the gastro-duodenal and inferior pancreatic arteries (two rats); the superior pancreatico-duodenal and inferior pancreatic arteries (one rat); the superior pancreatico-duodenal artery (ten rats); and the splenic vein (two rats). Eight rats were used as controls.

The pancreas was examined in all rats, either after arteriography and dissection or by histological methods.

RESULTS

Arterial pattern

The pancreas in the rat is of a diffuse dendritic type, extending from the duodenal loop into the gastro-splenic omentum. The chief part of this duodenal pancreas follows the curve of the gut but extends to a variable degree in its wide mesentery, increasing in extent with age. The tail is the thickest part of the pancreas.

The arterial and venous patterns have been described previously by Greene (1935). Her observations on venous drainage have been confirmed in this investigation, and therefore only a brief reference will be made to venous pattern. It is necessary, however, to describe the arterial supply in greater detail, with particular reference to anastomotic channels.

The arterial supply is derived from two sources only, the coeliac and superior mesenteric arteries (Pl. 1, fig. 1). Both arteries are unpaired and arise from the ventral surface of the aorta. The coeliac artery is at the level of the crus of the diaphragm and has a short trunk (less than 0·5 cm. in length), which extends downwards and to the left. Of the two arteries, the superior mesenteric is the larger; arising below the coeliac artery it crosses to the right, ventral to the inferior vena cava, the superior mesenteric vein, and the beginning of the portal vein; it then courses downwards and forwards in the dorsal surface of the pancreas, closely applied to it and lying inferior to the transverse colon.

The coeliac artery divides into three branches, the splenic, the left gastric and the hepatic arteries. The splenic artery is the largest branch and appears to be a direct continuation of the coeliac artery; it passes downwards and to the left behind the stomach and ends by dividing into five to seven splenic branches, its terminal portion running on to the greater curvature of the stomach as the left gastro-epiploic artery. Throughout its course the splenic artery is embedded in the dorsal surface of the pancreas. The first splenic branch arises close to the coeliac artery and runs towards the inferior pole of the spleen; its course is parallel to that of the parent vessel but inferior to it. At the origin of this vessel there are always two or more lymph nodes which are a useful guide during operative procedures. A smaller and inconstant artery occasionally arises above these nodes and runs towards the cardiac opening of the stomach. A slender arterial loop (the 'splenic loop') joins the proximal third of the splenic artery to the junction of the proximal and middle, or middle and distal thirds. This loop is only evident after ligature and division of the main trunk (Pl. 1, fig. 2).

The left gastric artery at its origin turns immediately towards the cardiac opening of the stomach, from which it branches to supply both surfaces. One small branch leaves the artery just below the division and runs along the lesser curvature towards the pylorus and the first part of the duodenum (Pl. 1, fig. 1). This vessel is of some importance since, after ligature and division of either the gastro-duodenal or the superior pancreatico-duodenal arteries, it dilates and provides an anastomosis between the latter artery and the right gastro-epiploic artery as it crosses the duodenum.

From its origin the hepatic artery turns to the right and passes upwards and backwards towards the porta hepatis. As it crosses behind the pylorus and passes just above the first part of the duodenum it sends a branch forwards which runs medial to the ductus choledochus. This is the gastro-duodenal artery, which is embedded in the dorsal surface of the pancreas. It is a short vessel, less than 0·5 cm. in length, and divides into the superior pancreatico-duodenal and right gastro-epiploic arteries. The latter runs along the greater curvature of the stomach and anastomoses with its fellow of the left side. The superior pancreatico-duodenal artery follows that portion of the pancreas which lies under cover of the first part of the duodenum and passes in its substance to the right, where it anastomoses with the superior division of the inferior pancreatico-duodenal artery at the junction of the first and second parts of the duodenum.

Of the two pancreatic branches of the superior mesenteric artery, the inferior pancreatico-duodenal and the inferior pancreatic arteries, the former is the larger. These arteries may arise singly or by a common stem; if the latter, the origin is from the inferior aspect of the superior mesenteric artery. Usually the inferior pancreatico-duodenal artery arises alone from the superior aspect of the superior mesenteric artery; it runs upwards and to the right, dorsal to the transverse colon, at the superior border of which it bifurcates into superior and inferior divisions. The inferior division runs along the medial border of the second part of the duodenum to the junction of the second and third parts where it anastomoses with the inferior pancreatic artery.

In the head of the pancreas slender vessels form a delicate branching network joining the superior pancreatico-duodenal artery to the superior division of the inferior pancreatic artery. These filigree branches are more evident in mature animals (over 180 g. in weight), i.e. those with a pancreas ramifying widely in the duodenal mesentery, and are presumably more effective as anastomotic channels after ligature and division of the main pathways. Fine vessels contributing to this network arise from the inferior pancreatico-duodenal artery before it divides.

The inferior pancreatic artery passes downwards and to the right to anastomose with the inferior division of the inferior pancreatico-duodenal artery.

Venous drainage

Several veins issuing from the spleen unite and run parallel with the splenic artery. The left gastro-epiploic vein, passing along the greater curvature of the stomach towards the hilum of the spleen, joins radicles of the splenic vein issuing from the hilum to form the origin of the splenic vein. Into this vein run pancreatic veins from the tail of the pancreas and also the short gastric veins which drain the fundus of the stomach. The coronary vein passes dorsal to the stomach, draining the area supplied by the left gastric artery, and enters the splenic vein.

Apart from receiving such tributaries as the middle colic, the inferior mesenteric, the right colic, the ileo-colic and a series of intestinal veins, the superior mesenteric vein also receives the inferior pancreatico-duodenal vein which drains the head and body of the gland.

The area supplied by the gastro-duodenal artery is drained by the pyloric vein. Starting below the pylorus by the union of small radicles it runs from left to right and in its course is joined by the superior pancreatico-duodenal vein. A short distance above the splenic vein and just below the liver the pyloric vein enters the portal vein.

In the dorsal surface of the head of the gland and in the duodenal mesentery the splenic and superior mesenteric veins unite to form the portal vein. It lies superior to the superior mesenteric artery and passes upwards to the liver.

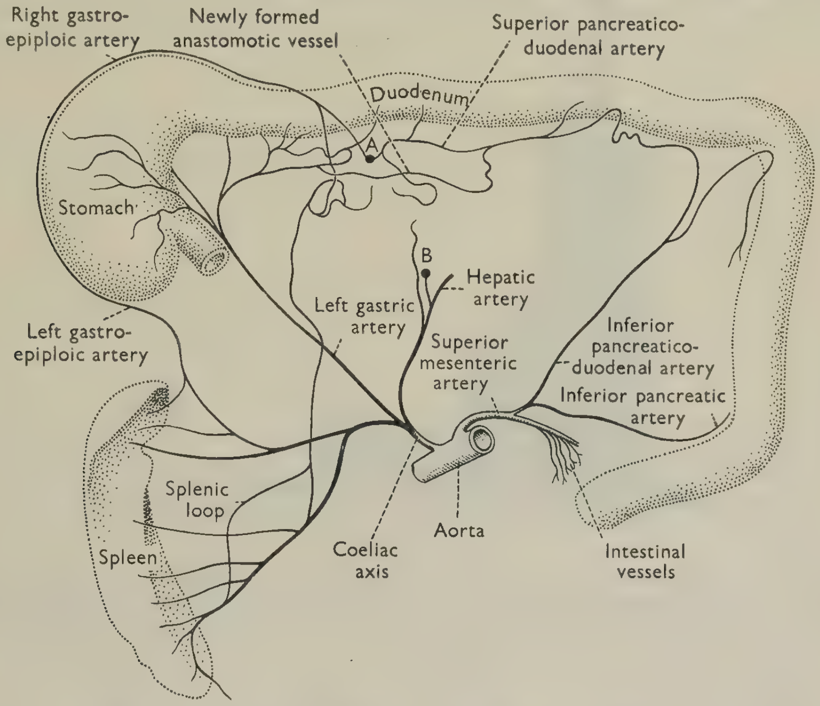
The collateral circulation as shown following experimental arterial interruption

As early as 2 days after interruption of the splenic artery and injection with 'Chlorbismol' a fine tortuous vessel, lying within the substance of the pancreas, is seen joining the right gastro-epiploic artery to the splenic loop. Additional subsidiary arterial anastomoses to the tail are provided from the left gastro-epiploic artery. These anastomotic channels are still present as long as 16 weeks following operation.

Three days following interruption of the gastro-duodenal artery two new channels are clearly visible (Text-fig. 1). The first of these is the vessel seen after interruption of the splenic artery; the second is a branch from the left gastric artery which anastomoses with the superior pancreatico-duodenal artery. These two channels can be seen 3 weeks after operation.

Interruption of the splenic, superior pancreatico-duodenal and inferior pancreatico-duodenal arteries, followed by injection 7 days later, reveals many new channels (Pl. 1, fig. 2). The tail of the pancreas is supplied by the coeliac artery through the splenic loop. Anastomoses from the left gastric artery run across the fundus of the stomach to join the splenic artery at its middle and distal thirds. To supply the area presumably rendered ischaemic by division of the superior and inferior pancreatico-duodenal arteries the inferior pancreatic artery dilates and extends along the medial side of the duodenum to the level of division of the inferior pancreatico-duodenal artery. There are a number of fine vessels in this area which join one another and eventually form a single channel which anastomoses with the superior pancreatico-duodenal artery, above the site of ligature. No anastomosing channels are seen about the origin of the inferior pancreatico-duodenal artery or even in its first part, i.e. before passing dorsal to the transverse colon. Finally the branch of the left gastric artery to the right gastro-epiploic artery dilates and anastomoses markedly with the latter. Three weeks after operation these channels are still visible.

Seven days after ligature and division of the splenic, the gastro-duodenal, the inferior pancreatico-duodenal and the inferior pancreatic arteries, there is a retrograde filling of vessels beyond the sites of interruption particularly in those animals injected after an interval of 6 or more days (Pl. 2, fig. 3). Anastomoses are clearly present in the pancreatic tail between the hepatic and splenic arteries, the left gastric and splenic arteries, and the right gastro-epiploic and splenic arteries. All these vessels are fine and slender but together form a firm anastomosis. In the body of the pancreas delicate branches unite the left gastric and right gastro-epiploic arteries to the superior pancreatico-duodenal. Twigs from the hepatic artery also join the superior pancreatico-duodenal artery. The inferior pancreatico-duodenal and inferior pancreatic arteries supplying the head of the pancreas are clearly visible; fine channels extend from the middle colic close to its origin and reach the second and third parts of the duodenum. The terminal branches of the superior pancreatico-duodenal artery and the superior division of the inferior pancreatico-duodenal artery are not evident. Three weeks after operation these anastomoses can still be seen.

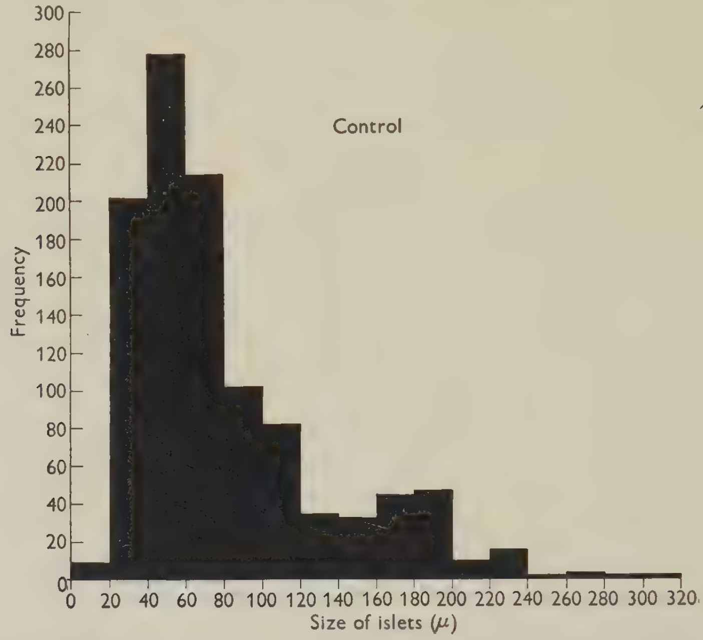

Right gastro-epiploic artery
Newly formed anastomotic vessel
Superior pancreatico-duodenal artery
Duodenum
A
Stomach
B
Hepatic artery
Left gastric artery
Superior mesenteric artery
Inferior pancreatico-duodenal artery
Left gastro-epiploic artery
Inferior pancreatic artery
Splenic loop
Aorta
Coeliac axis
Spleen
Intestinal vessels

Text-fig. 1. A diagram of the vascular channels supplying the rat pancreas as seen 3 days after ligature and division of the gastro-duodenal artery. *A* and *B* in the figure represent the sites of ligature of this vessel. The fine, tortuous anastomotic vessel joining the right gastro-epiploic artery to the splenic loop, which also becomes obvious following interruption of the splenic artery, is clearly shown. Also shown is the newly formed anastomotic vessel which appears following interruption of the gastro-duodenal artery.

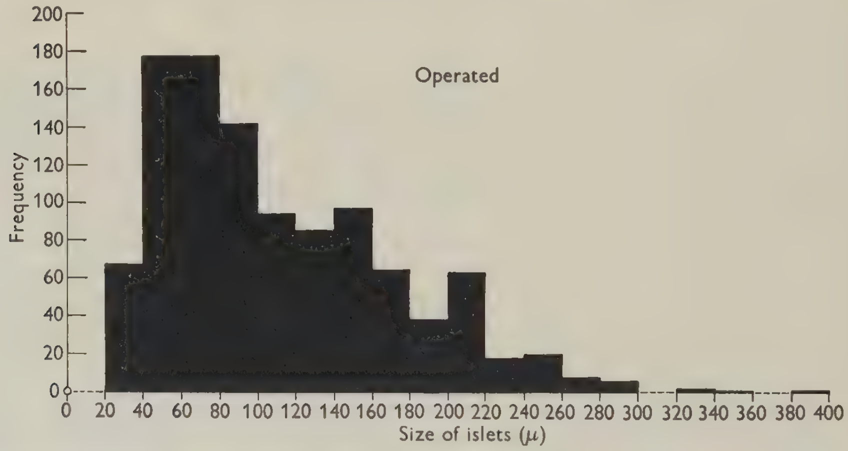
The effect of experimental arterial interruption on the pancreas

To determine the efficiency of the anastomosing channels, histological examination of the pancreas has been carried out following interruption of various arteries. Following formol-saline fixation, Ehrlich's haematoxylin and eosin was used, and after fixation in Helly's fluid, Gomori's (1939) method for α and β cells of the islets of Langerhans.

After interruption of the splenic artery and observation at intervals from 1 day to 16 weeks there are no macroscopic changes. Histologically the acini remain unchanged, but histograms of the diameter of the islets of Langerhans (Text-figs. 2 and 3) suggest that the islets of the operated animals show an increase in diameter.



Text-fig. 2. Histogram of the maximum diameters of the islets of Langerhans in four control rats, 260 islets being measured in the pancreas of each rat. Each rat was subjected to a sham abdominal operation, in which the pancreas was only handled, 3 days previously.



Text-fig. 3. Histogram of the maximum diameters of the islets of Langerhans in four experimental rats in which the splenic artery was divided 3 days previously. 260 islets were measured in the pancreas of each rat. Notice the apparent shift to the right.

This apparent increase is not significant statistically, however, as shown by the application of Students 't' test, the findings of which are summarized in Table 1, the rat, and not the islet being the unit of measurement. The α and β cells are normal in appearance. Correlated biochemical investigations confirm that the blood-sugar level in the experimental animals does not deviate from the controls.

Following interruption of three or more arteries patchy necrosis occurs throughout the pancreas, though it is very limited and by no means extensive. Macroscopically the pancreas loses its pink appearance and becomes dusky, suggesting venous congestion, but there is no obvious oedema or overall shrinkage of the pancreas. By the twenty-fourth day after operation the pancreas has regained its normal colour.

Table 1. *The mean diameter of the islets of Langerhans in* μ *in four control rats and four operated rats 3 days after interruption of the splenic artery*

Control	Operated
76·91	71·60
121·09	124·50
109·52	127·24
87·09	113·60
$\bar{x}$ = 98·65	$\bar{x}$ = 109·24
S.D. ± 19·95	S.D. ± 25·77
S.E. ± 10·01	S.E. ± 12·9
'*t*' = 0·6364	*P* = > 0·5

Table 2. *The number and diameter of islets and* α *and* β *cells per islet in* 46·705 *sq.mm.* of histological sections of the rat pancreas,* 4 μ *in thickness, following interruption of the gastro-duodenal, splenic, inferior pancreatico-duodenal and inferior pancreatic arteries*

(In each case the control animal was subjected to a sham abdominal operation in which the pancreas was only handled. The maximum diameter of each islet was measured, and the mean of these measurements calculated for each pancreas.)

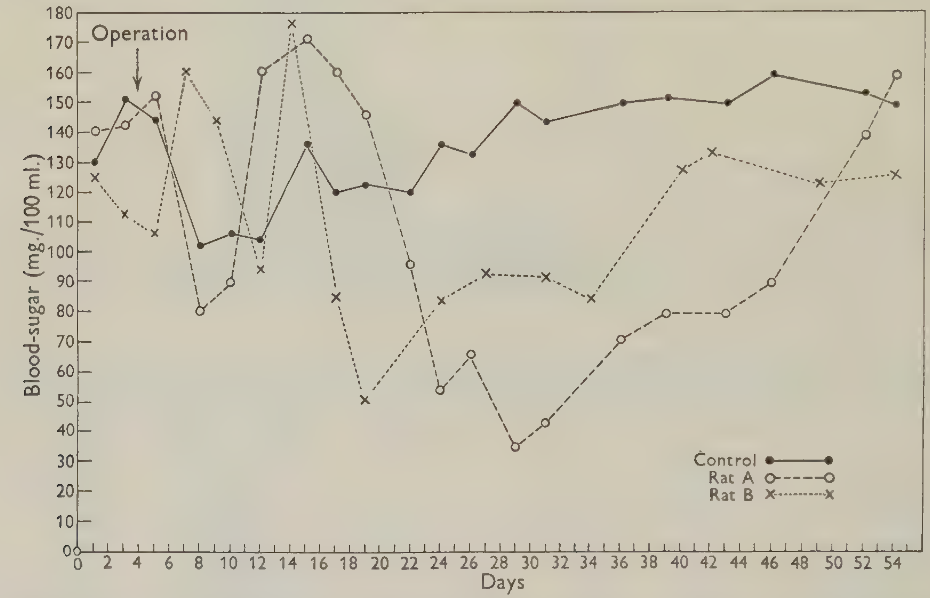
Animal	No. of days after operation	No. of islets	Mean diameter of islets in μ	Mean number of β cells per islet cross-section	Mean number of α cells per islet cross-section
Operated I	24	390	57	25·8	13·7
Control I	24	99	139	34·5	5·4
Operated II	28	384	64	27·8	8·7
Control II	28	102	121	36·0	5·9
Operated III	51	229	70	35·2	5·1
Control III	51	61	112	39·9	6·2
Operated IV	55	200	92	37·5	7·1
Control IV	55	74	144	39·2	6·0

* The measurements were made in each pancreas in an area of $\frac{\pi(133\cdot6/2)^2\times 50\times 6}{300^2} = 46\cdot705$ sq.mm.

where 133·6/2 = the radius of the magnified field on the projection screen.
50 = the number of fields per single histological section of pancreas examined at random.
6 = the number of 1 in 20 histological sections examined.
300 = the magnification on the projection screen.

Microscopically there are obvious changes in the acini. Most are normal but others show either a condensation of zymogen granules or complete disintegration of the cellular architecture. Fibrosis of the necrotic zones results and organization occurs within 6 days. In these areas there are only remnants of the islets, showing vacuolation and degranulation of the α and β cells; there is no evidence in these sites of islet regeneration.

Twenty-four to twenty-eight days after operation the changes in the islets of Langerhans in the areas free from necrosis are most striking. In those animals in which the gastro-duodenal, the splenic, the inferior pancreatico-duodenal and inferior pancreatic arteries have been ligated, small collections of β cells can be seen, which are clearly new islets developing from the acini. In some cases only two or three β cells can be seen in intimate contact with the acini (Pl. 3, fig. 4), which may have spurs and processes of cytoplasm infiltrating between the cells. The β cells at the periphery of larger islets are in direct contact with the cells of some of the neighbouring acini (Pl. 3, figs. 5 and 6), and acinar nuclei with portions of surrounding cytoplasm are passing into adjacent islets or inter-acinar spaces, with marked change in the staining properties of the cytoplasmic granules, so as to take on the appearance of β cells (Pl. 3, fig. 5). There is an absence of α cells in the small islets, but other larger islets do possess them (Pl. 3, fig. 6), often in large numbers.



Text-fig. 4. Graph showing the level of blood-sugar in two rats (A and B) following interruption of the gastro-duodenal, splenic, inferior pancreatico-duodenal and inferior pancreatic arteries, and in a control rat in which the pancreas was only handled at operation.

There is no capsule around the new 'islets' of β cells, the cells themselves being histologically normal. Table 2 summarizes the findings in a quantitative investigation of the number and diameter of islets, and the mean number of α and β cells in each islet.

All of the operated animals show an increase in the number of islets over the control rats; there is also a small decrease in the mean number of β cells per islet, but their absolute number is obviously greater than that in the control animals. Operated animals I and II show a uniform slight increase in the number of α cells per islet, but the most pronounced change is the marked decrease in the mean diameter of the islets in these two animals, suggesting an increase in the production of new islets by acino-insular transformation. These changes are transient and slowly normality is approached.

An investigation designed to determine the relationship between these histological changes and physiological function was undertaken in order to determine their significance. Using a Mantoux syringe, 0·1 ml. of whole blood was removed by cardiac puncture in control and experimental animals following interruption of the gastro-duodenal, the splenic, the inferior pancreatico-duodenal and inferior pancreatic arteries. An estimation of the blood-sugar was made photo-colorimetrically by the method of King (1946). Text-fig. 4 shows the result of this investigation. Eighteen to thirty days after operation there is a depression of the level of blood-sugar to 40–60 mg. per 100 ml., coincident with the absolute increase in α and β cells. The newly formed β cells are therefore not only microscopically normal but also functional physiologically.

DISCUSSION

From the above observations it is evident that there is a firm anastomosis between the superior mesenteric and coeliac arteries. Degenerative changes do not develop until more than two arteries are divided; even division of the four main arterial paths (gastro-duodenal, splenic, inferior pancreatico-duodenal and inferior pancreatic arteries) produces only a temporary disturbance, as shown by the deviation of the blood-sugar level from normal. This is in marked contrast to the results obtained following interruption of the testicular (Harrison, 1949; Oettlé & Harrison, 1952) and adrenal arteries (Harrison, 1951). Haist (1944) claims that the insulin content of the pancreas increases with age. This factor may well be due to arteriosclerosis of the blood vessels of the pancreas. Further, it is possible that the sclerotic arterial changes in diabetes, which affect not only arteries and arterioles but also veins and capillaries (Root, 1949), may be in some measure a compensatory mechanism designed to increase the insulin output of the pancreas. The converse is also true, for the better the control of diabetes the less likely the incidence of vascular change (Millard & Root, 1948).

'The reaction of the tissues in different subjects varies with identical operative procedures. In some, even with the most gentle manipulation, vasoconstriction is marked, with at least a temporary circulatory embarrassment of the parts supplied and drained' (Baker & Evoy, 1942). It is reasonable to suggest, therefore, that at least some of the immediate post-operative complications associated with surgery of the abdomen (in particular splenectomy, resection of the head of the pancreas and gastrectomy) follow interference with the blood supply of the pancreas.

There is no uniform agreement as to the optimum site of ligature of the splenic artery during splenectomy. Ligature of the splenic artery at its origin from the coeliac axis, not only interrupts blood flow through the short gastric arteries, but deprives the pancreas of its splenic arterial branches; the above experiments suggest that this procedure would be unlikely to produce permanent hypoglycaemia.

The concept of acino-insular transformation in the adult differentiated pancreas has many supporters (Laguesse, 1905; Hughes, 1947; and others). The transformation of acinus cells into islet cells with loss of zymogen granules is clearly seen in the rat pancreas following ischaemia, as an exaggeration of the process occurring in the normal, adult pancreas. This accentuation makes the transformation more obvious histologically, and is responsible for the increase in number of islets and the decrease in mean diameter of the islets noted in this investigation 24–28 days after the production of ischaemia, thus suggesting an increase in the number of newly formed islets. This is confirmed by the coincident depression of blood-sugar. Careful search failed to demonstrate formation of islets from anything but acini; no evidence could be found, for example, of formation of β cells by proliferation of duct epithelium. In several situations β cells could be found being produced from acini in the neighbourhood of a duct (Pl. 3, fig. 7), which appeared normal in histological structure, and showed no connexion with the β cells when examined in serial sections. The possibility of coincident processes of genesis of islets, although not completely excluded, therefore appears to be less likely in the ischaemic pancreas.

SUMMARY

The arterial supply of the pancreas in the albino rat is derived from the artery of the fore-gut and the artery of the mid-gut, the coeliac and superior mesenteric arteries. The anastomosis between branches of these two vessels is so efficient that clearly demarcated areas of necrosis corresponding to the territories of arterial distribution do not result from their interruption. Interruption of all four main pancreatic arteries does, however, result in an increase in the number of islets of Langerhans. The newly formed islets are functional and responsible for a transient hypoglycaemia.

The clinical implication of these findings is discussed.

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REFERENCES

Baker, J. W. & Evoy, M. M. (1942). Insult to the testis in herniorrhaphy. *Surg. Gynec. Obstet.* 75, 285–288.

Gomori, G. (1939). A differential stain for cell types in the pancreatic islets. *Amer. J. Path.* **15**, 497–500.

Greene, Eunice C. (1935). Anatomy of the rat. *Trans. Amer. Phil. Soc.* **27**, I–XI, 1–370.

Haist, R. E. (1944). Factors affecting the insulin content of the pancreas. *Physiol. Rev.* **24**, 409–444.

Harrison, R. G. (1949). The distribution of the vasal and cremasteric arteries to the testis and their functional importance. *J. Anat., Lond.*, **83**, 267–282.

Harrison, R. G. (1951). A comparative study of the vascularization of the adrenal gland in the rabbit, rat and cat. *J. Anat., Lond.*, **85**, 12–23.

Hughes, H. (1947). Cyclical changes in the islets of Langerhans in the rat pancreas. *J. Anat., Lond.*, **81**, 82–92.

King, E. J. (1946). Colorimetric method for 'true sugar' in 0·05 ml. of blood. *Micro-Analysis in Medical Biochemistry*, pp. 20–23. London: J. and A. Churchill Ltd.

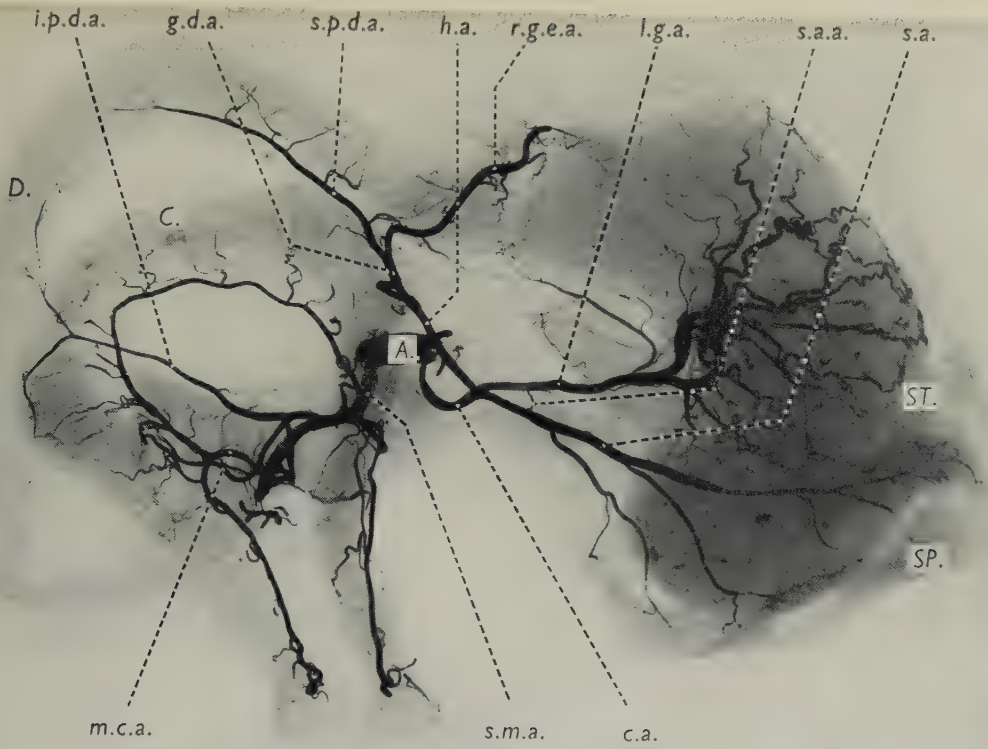
Laguesse, M. E. (1905). Ilots endocrines et formes de transition dans le lobule pancréatique (homme). *C.R. Soc. Biol., Paris*, **58**, 542–544.

Millard, E. B. & Root, H. F. (1948). Degenerative vascular lesions and diabetes mellitus. *Amer. J. dig. Dis.* **15**, 41–51.

Oettlé, A. G. & Harrison, R. G. (1952). The histological changes produced in the rat testis by temporary and permanent occlusion of the testicular artery. *J. Path. Bact.* **64**, 273–297.

Root, H. F. (1949). Diabetes and vascular disease in youth. *Amer. J. med. Sci.* **217**, 545–553.

Scharrer, E. (1940). Arteries and veins in the mammalian brain. *Anat. Rec.* **78**, 173–196.



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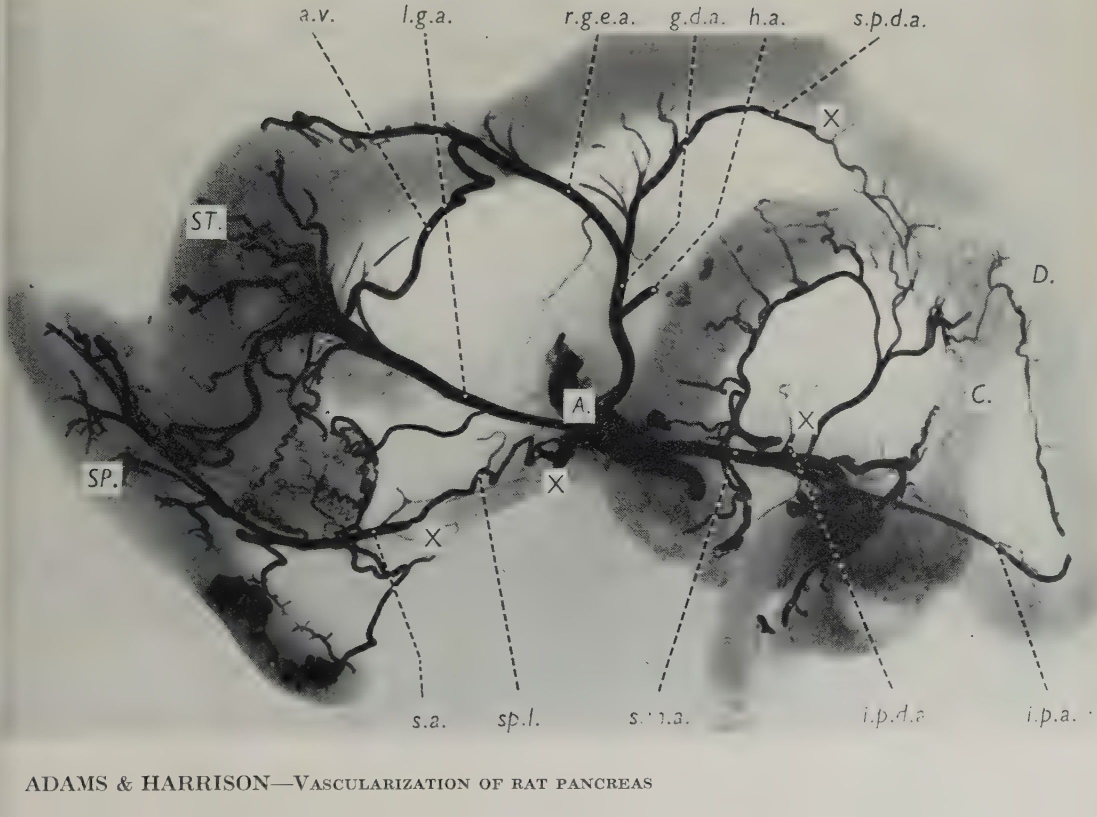

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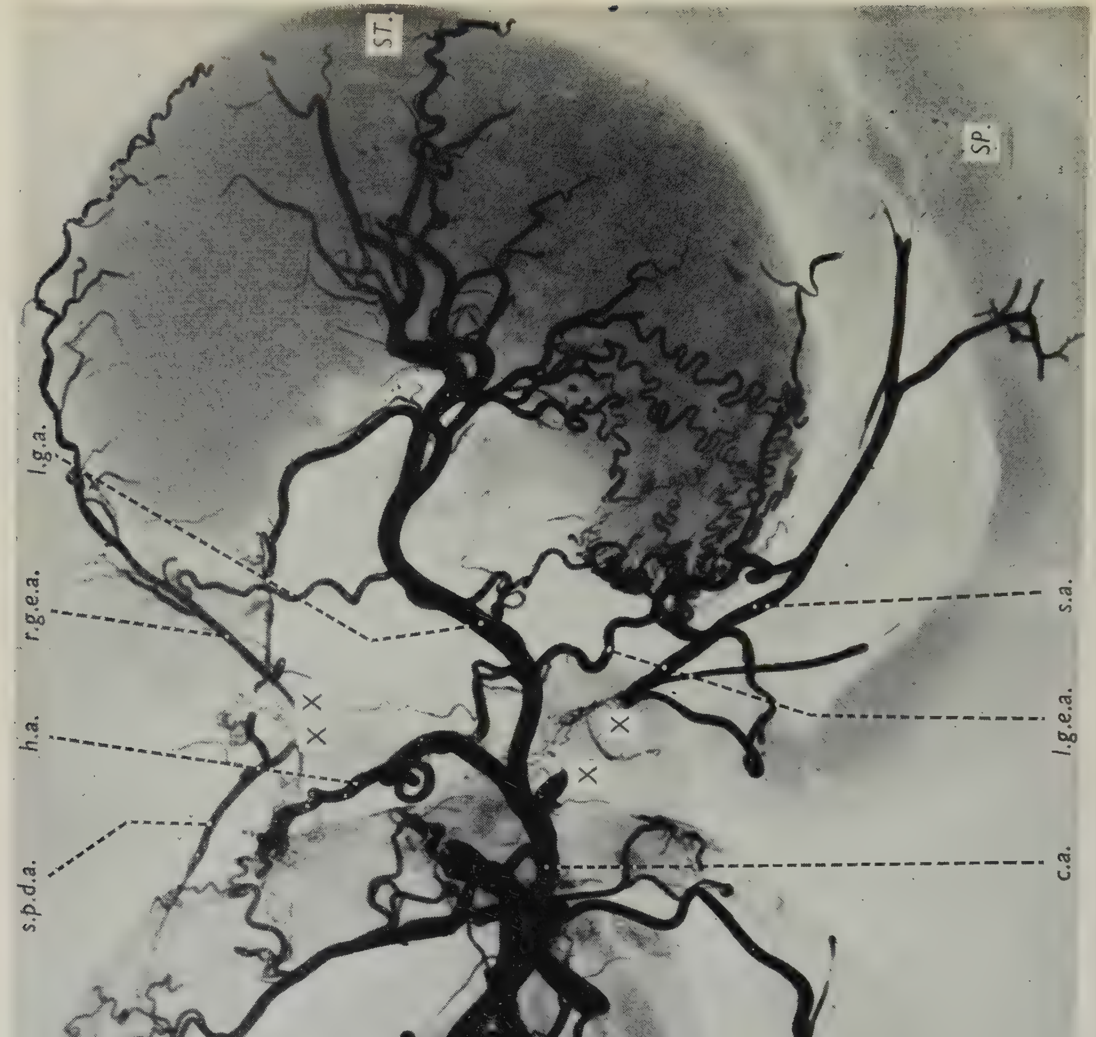
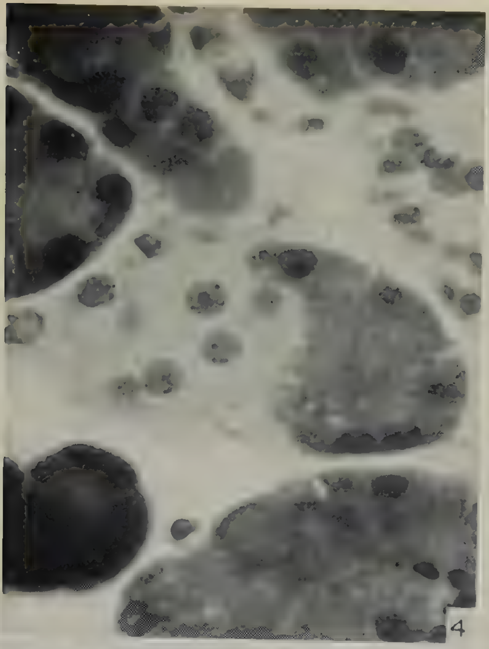
ADAMS & HARRISON—Vascularization of rat pancreas



ADAMS & HARRISON—VASCULARIZATION OF RAT PANCREAS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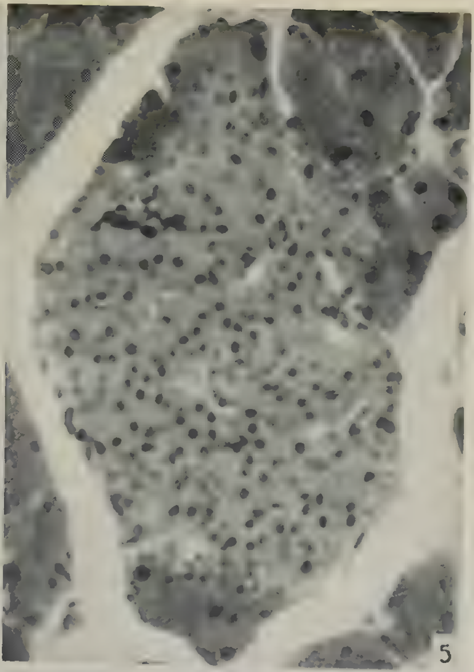
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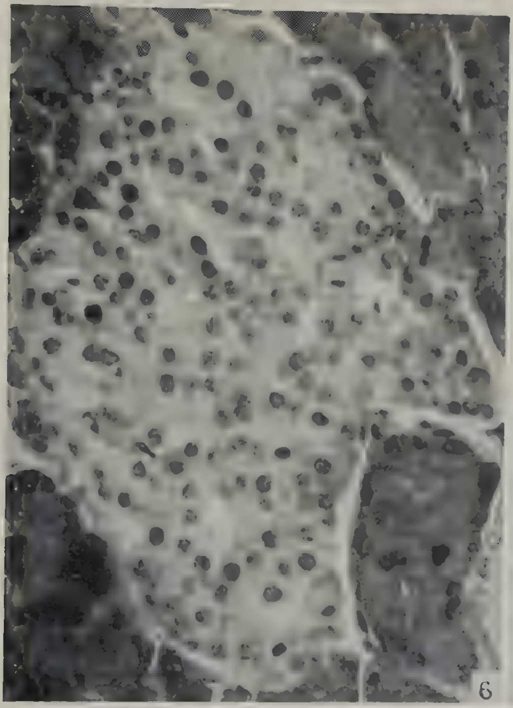
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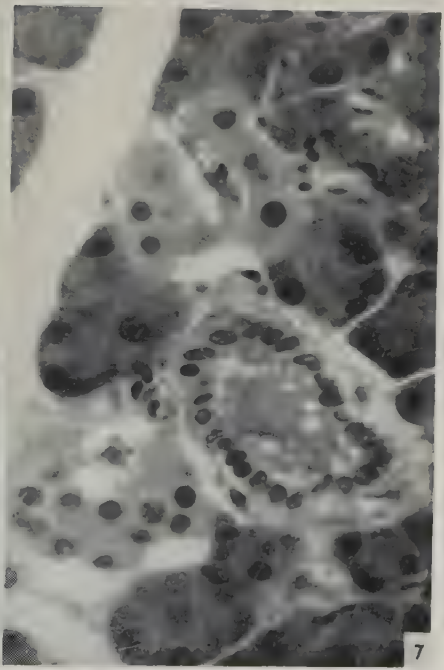
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ADAMS & HARRISON—Vascularization of rat pancreas

EXPLANATION OF PLATES

PLATE 1

Fig. 1. The normal arterial pattern of the rat pancreas as visualized from the anterior aspect following injection with 10% 'Chlorbismol' through the thoracic aorta immediately after death and radiography on Kodaline film. The viscera were removed from the animal before radiography. The anastomosis between the superior pancreatico-duodenal artery and the superior division of the inferior pancreatico-duodenal artery can be seen. The inferior pancreatic artery, and the anastomosis between this and the inferior division of the inferior pancreatico-duodenal artery, are not shown in the figure. (×2·5.)

Fig. 2. The arterial pattern of the rat pancreas, as seen from the posterior aspect, illustrating the collateral circulation 7 days after ligature and division of the superior pancreatico-duodenal, inferior pancreatico-duodenal and splenic arteries. The vessels were injected with 10% 'Chlorbismol' and radiographed on Kodaline film. The inferior pancreatic artery is now clearly seen since it has taken over the arterial supply of the part of the pancreas rendered ischaemic by division of the superior and inferior pancreatico-duodenal arteries. (×2.)

PLATE 2

Fig. 3. Radiograph of the arterial pattern of a rat pancreas 7 days after ligature and division of the splenic, gastro-duodenal, inferior pancreatico-duodenal and inferior pancreatic arteries. Radiography on Kodaline film after injection of 10% 'Chlorbismol' through the thoracic aorta. The inferior pancreatic artery is clearly seen because of retrograde filling of this vessel through its anastomoses with the inferior pancreatico-duodenal and middle colic arteries. Many fine anastomotic channels are present, and are described in the text. Note the well-marked anastomotic vessel passing from the right gastro-epiploic artery, behind the left gastric artery to the splenic artery. (×5.)

Key to Lettering on Plates 1 *and* 2

A.	aorta
a.v.	anastomotic vessel from left gastric artery to right gastro-epiploic artery
C.	colon
c.a.	coeliac artery
D.	duodenum
g.d.a.	gastro-duodenal artery
h.a.	hepatic artery
i.p.a.	inferior pancreatic artery
i.p.d.a.	inferior pancreatico-duodenal artery
l.g.a.	left gastric artery
l.g.e.a.	left gastro-epiploic artery
m.c.a.	middle colic artery
r.g.e.a.	right gastro-epiploic artery
s.a.	splenic artery
s.a.a.	small, inconstant, anastomotic artery
s.m.a.	superior mesenteric artery
SP.	spleen
s.p.d.a.	superior pancreatico-duodenal artery
sp.l.	splenic loop
ST.	stomach
X.	site of ligature and division of an artery

PLATE 3

The figures on this plate are photomicrographs of histological sections of the pancreas twenty-four (Figs. 4 and 7) and twenty-eight (Figs. 5 and 6) days after ligation of the splenic, gastro-duodenal, inferior pancreatico-duodenal and inferior pancreatic arteries.

Fig. 4. A small collection of β cells in intimate contact with a neighbouring acinus. The β cells have no surrounding capsule, and appear to be produced by transformation of the acinar cells. (×774.)

Fig. 5. A large islet of Langerhans whose peripheral β cells (at the top and bottom of the islet) appear to be produced by direct transformation from acinar tissue. This islet contains no α cells. (×323.)

Fig. 6. A large islet in which the β cells are in direct contact with acinar cells (at the top left-hand corner of the islet). There are some cells along the right-hand border of the islet (darker cells in the figure) which have the staining properties of α cells with Gomori's (1939) method. (×496.)

Fig. 7. Two small collections of β cells in the neighbourhood of a duct. Those above the duct in the figure appear to be in the process of transformation from acini, but have no connexion with the duct. The β cells below and to the left of the duct are also in contact with an acinus at one point, but there is no indication of active proliferation of duct epithelium, which has a normal histological appearance. (×731.)

IMPREGNATION AND DEVELOPMENT IN SILVER STAINING

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In spite of the multiplicity of silver solutions and developing agents and the varied manner of their employment, the many different silver staining techniques for nervous tissues are capricious. This appears to be due in part to an inadequate understanding of the processes involved in impregnation and development. It was the purpose of this research to elucidate with greater exactitude the properties of the impregnating solution and the reactions occurring during the development phase of silver staining.

The investigations of Foot (1929) established the importance of controlling the silver concentration and pH of the silver bath for successful staining, and Holmes (1943), recognizing that the significant variables in the silver solution used in Bodian's method (1936) were the silver and hydrogen-ion concentrations, stained nerve fibres in paraffin sections with dilute silver nitrate solutions adjusted to a particular pH. Pearson & O'Neill (1946) and Romanes (1950) also elaborated methods, using acid or weak alkaline solutions of silver salts. These techniques, using very different impregnating solutions, were designed for tissues fixed in a similar manner. Consequently, there appeared to be some relationship between the pH, the silver concentration, the time, and the temperature of the incubation, for when suitably varied one with another they produced similar results. A relationship has been postulated, without direct experimental proof, by Kubie & Davidson (1928) and Kubie (1929) who stated that the differences in the activity of the silver salts (they used ammoniacal silver salts) must be compensated for by altering the concentration, duration, and temperature of the silver bath and the strength of the developer. But there was no evidence as to why, or how, this compensation took place, nor why certain hydrogen- and silver-ion concentrations were necessary in different methods, which, if departed from, resulted in failure of the staining process. Information was equally deficient regarding the action of the developer. Silver (1942) believed the pH of the developer to be all-important in the staining process, but Davenport, McArthur & Bruesch (1939) found that the pH of an amidol developer had little effect on the staining of paraffin sections impregnated in a protargol solution, except when it approached the isoelectric point of protargol. Holmes (1943) concurred with this view, but disagreed with Kubie's (1929) opinion that diffusion of silver from paraffin sections affected the specificity of the stain, and believed that differences in staining produced by different developers was due only to the more complete development of the silver in the section when the more active developers were used.

EXPERIMENTAL PROCEDURE

Before the present investigation could be undertaken it was essential to develop a reliable silver staining method which permitted complete control of the chemical and physical factors involved. This proved singularly difficult, but the following method was ultimately evolved from the Holmes (1943) technique. Dewaxed sections were:

(1) Washed in running water for 2 hr.

(2) Rinsed in three changes of distilled water.

(3) Placed in a slide bath containing borax-boric acid buffer solution (10 c.c. of buffer–90 c.c. distilled water)* and incubated. A flask containing 99 c.c. of a similar solution was also placed in the incubator. When the solutions in the flask and bath were stabilized at a temperature of 53·5° C., 1 c.c. of 1 % silver nitrate solution was added to the buffer within the flask and thoroughly mixed. The buffer was decanted rapidly from the slides and the silver buffer solution added to them. The impregnation was continued for a predetermined time at the same temperature.

(4) The sections were then developed.

(5) In some instances, the sections were toned in 0·2 % gold chloride for 10 min., washed with distilled water for 1 min., reduced in 2 % oxalic acid for 5 min., washed for 15 min., and finally dehydrated and mounted.

The term 'impregnation' is reserved for the reactions taking place between the section and the buffered silver nitrate solution. The term 'staining' refers to the final appearance of the section when cleared and mounted.

The impregnation method described possesses certain advantages. The time taken for solutions in the incubator to reach a stable level varies in different types of slide baths and in different parts of the incubator, and if the sections and solutions are allowed to reach a stable temperature before the silver is added this ensures:

(*a*) Precise control of the duration and therefore of the amount of reaction between the section and the silver nitrate at a constant temperature.

(*b*) That the sections are always in equilibrium with their chemical environment before reacting with the silver. This is important, for temperature variation causes variation in pH and if the pH of the solution affects the silver-reacting qualities of the section, it is essential that this should be constant for any particular pH.

The following experiments were carried out on 10 μ paraffin sections of sympathetic ganglia and spinal cord of rabbits fixed in the solution of Davenport & Kline (1938) consisting of formic acid 5 c.c., trichloracetic acid 5 g., *n*-propyl alcohol 25 c.c., *n*-butyl alcohol 65 c.c., dehydrated in ethyl and butyl alcohol and imbedded in paraffin.

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* The buffer solutions consisted of an M/20 borax solution containing 19·1 g. $Na_2B_4O_7 . 10H_2O$ in 1 l., and an M/5 boric acid solution containing 12·4 g. H_3BO_3 in 1 l. For a particular pH the solutions were mixed in the proportions given in the Palitzsch buffer table. The dilution of the buffer and the omission of the sodium chloride from the boric acid solution caused insignificant differences in the value of the pH as given in the table and these have been ignored.

THE PROPERTIES OF THE IMPREGNATING SOLUTION

Hydrogen-ion concentration and the effects of varying the time of impregnation

Series of sections were impregnated in solutions containing 1 c.c. of 1 % silver nitrate buffered at pH 6·8, 7·4, 7·8, 8·2, 8·4, 8·7 and 9·1 for periods of 7 min., 2½ hr., and 24 hr. They were then developed in 1 % hydroquinone in a 10 % sodium sulphite solution and toned.

Seven min. impregnation. At pH 6·8, the section was covered with a grey granular precipitate and very faint neuronal staining was observed (Pl. 1, fig. 1). As the pH was raised to 7·8 the precipitate decreased markedly and some axonal staining was apparent, together with a general improvement in the definition of the neurons. With further increases in pH this improvement continued, until at pH 8·7 (Pl. 1, fig. 2) the cells and nucleoli were sharply stained in contrast to the clear background in which well-stained axons were visible; at pH 9·1 the staining became so heavy and diffuse that much of the contrast between the different constituents of the section was lost (Pl. 1, fig. 3).

Two and a half hours' impregnation. The most marked changes occurred at the extremes of the pH range. In the sections impregnated at pH 6·8 (Pl. 1, fig. 4) the axons, the neurons, and their nuclei, were now heavily stained, but at pH 9·1 the section was uniformly and evenly stained reddish brown in colour and had an undifferentiated flat appearance. The axons were no longer black as in the 7 min. impregnation, but were the same colour as the rest of the tissues and were scarcely distinguishable.

Twenty-four hours' impregnation. On removal from the silver bath the sections were a brown colour, which became intensified as the pH of the buffered silver solution increased. Microscopic examination of these undeveloped sections impregnated at high pH levels showed deep capsular staining and the cell cytoplasm was pale brown. Nuclear detail was evident, particularly at the periphery of the section, where pale brown axons could also be seen. When the sections impregnated at pH 6·8–7·8 were developed and toned, the axons were delicately stained in a feebly stained background; the neurons and nuclei too were sharply differentiated. Above pH 7·8, however, the sections had a flat undifferentiated appearance in which all structures were uniformly stained a reddish brown colour. Thus, whereas in the shorter impregnations of 7 min. and 2½ hr., the undifferentiated staining appeared only at the upper limit of the pH range, when the impregnation was prolonged for 24 hr., sections impregnated at much lower pH levels became evenly stained.

Progressive staining

Since the increase in incubation time to 2½ hr. resulted in the staining of the axons at pH 6·8, sections treated in this way were removed from the silver bath at 15 min. intervals, developed, and toned in order to study the progress of axonal staining.

After 15 min. the sections were almost completely unstained. With further impregnation, axonal staining commenced, and after 45 min. (Pl. 1, fig. 5) a coarse granular precipitate was distributed along the axons. This gradually increased in amount, and half an hour later the axons were clearly visible but still exhibited granular staining which had also appeared in the cell cytoplasm and nucleus (Pl. 1, fig. 6). With a further increase in incubation time to 1 hr. 45 min., the axons became heavily but unevenly stained; they had begun to lose their granular appearance and neuronal staining was more complete (Pl. 1, fig. 7). After 2½ hr. impregnation, the axons were evenly and regularly stained in sharp contrast to the faintly stained background tissue. The neurones were completely stained and considerable nuclear detail could be seen (Pl. 1, fig. 4).

Silver concentration

To evaluate the role of the silver-ion concentration, buffered silver nitrate solutions at pH 6·8 containing increasing amounts, 0·5–10 c.c. of 1 % silver nitrate, were added to sections. These were then impregnated for 15 min., and then developed and toned as before. The amount of distilled water added to the 10 c.c. of buffer solution was adjusted so that the final volume was always 100 c.c.

When 0·5–1 c.c. of silver nitrate was added no staining occurred. As larger amounts were added the staining of the axons gradually increased, closely followed by increased staining in the neurons. After the addition of 10 c.c. of silver nitrate the cells and axons were clearly stained (Pl. 1, fig. 8). The improvement in staining generally followed a course very similar to that seen when sections were impregnated at pH 6·8 for increasing lengths of time.

THE ROLE OF THE DEVELOPER

The effects of different developers

Slides were immersed in buffered silver solutions at pH 6·8, 7·4, 7·8, 8·0, 8·7, 9·1, at 53·5° C. for periods of 15 min., 1 and 2½ hr., and then rinsed and developed in one of the following solutions at 20° C. for 5 or 10 min.

(*a*)	Hydroquinone	1 g.	(*b*)	Hydroquinone	1 g.
	Sodium sulphite	10 g.		Distilled water	100 c.c.
	Distilled water	100 c.c.			
(*c*)	Amidol	0·5 g.	(*d*)	Amidol	1 g.
	Sodium sulphite	10 g.		Sodium sulphite	10 g.
	Sodium bisulphite	2·5 g.		Distilled water	100 c.c.
	Distilled water	100 c.c.			

It is unnecessary to give a detailed description of the staining produced by each developer after each impregnation, and the discussion will be confined to the most significant aspects of the results.

As the pH was raised and/or the time of impregnation was prolonged, it was found that under similar conditions staining first appeared with the amidol sulphite developer, secondly with the hydroquinone solution, thirdly with the amidol bisulphite mixture, and lastly with the hydroquinone sulphite solution. For every equivalent pH and time of impregnation the staining intensity of the section as a whole also increased in that order. But every developer had its own staining spectrum, for apart from the difference in staining intensity the same structures were stained to varying extents in each developer. This may be exemplified by sections of sympathetic ganglia impregnated at pH 7·4 for 1 hr. and developed in

1 % amidol sulphite, and in the 1 % hydroquinone sulphite developer—i.e. the most and least active developer used. In the former the cytoplasm of the neurons was deeply and evenly stained, the nuclear detail was almost complete and the axons were barely visible in the diffusely stained background tissue (Pl. 2, fig. 9). In the section developed in the hydroquinone sulphite solution the neuronal cytoplasm was irregularly stained with little nuclear detail and the finely stained blue-black axons were in marked contrast to the clear background (Pl. 2, fig. 10). These differences were apparent over the entire pH range. The hydroquinone developed sections resembled the sections developed with the amidol sulphite solution, but were paler and more granular; the amidol bisulphite caused staining which resembled that produced by the hydroquinone sulphite, but with more intense staining of cells and background and the axons had a finer appearance.

Each developer produced different effects, and the more active and faster the developer the more diffuse and even was the staining, the slower developer giving a more precisely differentiated axonal stain. It appeared that the speed of development played an important role. But it was not only a question of the rate of development, because prolonged immersion in the slow hydroquinone sulphite did not produce the staining comparable with the rapid amidol sulphite developer. Why did similarly impregnated sections stain so differently with different developers? It did not appear, as Holmes (1943) believed, that the hydroquinone sulphite developed only a proportion of the silver in the section as compared with the amidol sulphite solution. For, when stained, the chromidial bodies in the neurons, and the axons in the spinal cord and ganglia, were always more heavily stained and clearly differentiated after hydroquinone sulphite, whereas they should have been less heavily stained if development was incomplete. These considerations suggested further experiments.

Amidol sulphite and hydroquinone sulphite development

To determine whether the differences in the appearance of the sections were due to complete development in the amidol sulphite solution and incomplete development in the hydroquinone sulphite solution, sections were impregnated at pH 7·4, and 7·8, at 53·5° C. for 1 and 2½ hr., as before. From each bath, sections were placed directly, without rinsing to avoid all possible interference with the sections, in a variety of solutions:

(1) 1 % hydroquinone in 10 % sodium sulphite at 20° C. for 5 min.;
or (2) 1 % amidol in 10 % sodium sulphite at 20° C. for 5 min.;
or (3) 1 % hydroquinone in 10 % sodium sulphite at 20° C. for 5 min., and then rinsed in a similar solution before being transferred to a 1 % amidol in 10 % sodium sulphite at 20 % C. for 5 min.;
or (4) distilled water at 20° C. for 5 min. followed by immersion in 1 % amidol in 10 % sodium sulphite at 20° C. for 5 min.

If no silver was removed in the hydroquinone sulphite developer, a section placed in the hydroquinone sulphite and then in the amidol sulphite solution should resemble a section placed directly in amidol sulphite. Sections impregnated at pH 7·4 for 1 hr. and subjected to the double development procedure did not resemble sections placed directly in the amidol sulphite solution (Pl. 2, fig. 9). Their appearances were those of sections developed in the hydroquinone sulphite developer above (Pl. 2, fig. 10). The control sections placed in distilled water, though paler, were stained like those placed directly in the amidol developer (Pl. 2, fig. 9).

It was clear that the differences between sections developed in the hydroquinone and amidol sulphite developers were not due to diffusion of silver from the section in the solvent of the developer; but a definite loss of silver from the section had taken place, for after double development, no, or little, further development ensued in the amidol developer. Davenport & Kline (1938) observed that increasing the concentration of sodium sulphite in the developer increased differential staining, i.e. it produced an increased 'hydroquinone sulphite' effect. It was considered possible that the sulphite constituent could be responsible for the differences in the staining of the sections reduced in the hydroquinone and amidol sulphite developers.

The action of the sulphite in the developer

Sections impregnated at pH 7·8 and 8·5 for 1 and 2½ hr. were treated with sulphite solutions whose concentrations ranged from 0·25 to 10 %, for times varying between 15 sec. and 10 min., and then developed either in a 1 % amidol in 10 % sodium sulphite solution or a 1 % hydroquinone in 10 % sodium sulphite solution.

The sections of spinal cord impregnated at pH 7·8 for 2½ hr. and developed in 1 % amidol in 10 % sodium sulphite solution evidenced less staining after treatment with 1 % sodium sulphite for 1 min. than when an 0·25 % sodium sulphite solution was used. In sections of ganglia impregnated at pH 8·5 for 1 hr. and developed in 1 % hydroquinone in 10 % sodium sulphite the axons and cells were completely stained (Pl. 2, fig. 11). After 15 sec. in 2·5 % sodium sulphite cellular staining had diminished, nuclear detail was almost absent, but the axons were clearly visible in the colourless background (Pl. 2, fig. 12). After 2 min. in the sodium sulphite the sections were completely unstained (Pl. 2, fig. 13).

The evident correlation between staining intensity and the time in, and concentration of, the sulphite before development suggested that the difference in the appearance of amidol sulphite and hydroquinone sulphite developed sections could be partially attributed to the sulphite constituent of the developer. The complete absence of staining following immersion in 2·5 % sodium after 2 min. showed that the sulphite removed the silver which would have been reduced and deposited in the section by the developer, i.e. it removed the reducible silver on which the staining of the tissues depended. In the sulphite developer solution, therefore, the sulphite competed with the developing agent and tended to remove the reducible silver from the section. Amidol, a powerful and rapid developer, outpaced the sulphite and developed the silver *in situ*. Hydroquinone, a slower developer, was partially outpaced by the sulphite which removed some of the reducible silver in the section.

Further experiments confirmed this conception of developer sulphite competition for the available silver, and established that a low pH, apart from depressing the activity of the developing agent, also facilitated the removal of the silver by the sulphite, and vice versa.

DISCUSSION

It is evident from the sections stained for increasing lengths of time at pH 6·8 that nerve staining is not an end-point phenomenon. It takes place after a certain amount of reaction between the section and silver solution. To render the axons visible a certain quantity of silver must be deposited in them, and this amount can be deposited under various conditions of impregnation, providing they possess a certain degree of affinity for silver. If it is assumed that the pH controls their silver affinity, the results of the experiments fall into an intelligible pattern. As the pH increases up to pH 8·2, 8·4, the silver affinity of the axons increases and thus a shorter time of impregnation is needed for an adequate amount of reaction and silver deposition. At lower pH levels the axons have less affinity for silver and a longer impregnation time is required. However, increasing the silver nitrate concentration tenfold shortens the impregnation at pH 6·8 from 2½ hr. (Pl. 1, fig. 4) to 15 min. (Pl. 1, fig. 8). Though a section impregnated at a low pH and high silver concentration (Pl. 1, fig. 8) resembles a section impregnated at high pH and low silver concentration (Pl. 1, fig. 2), there are important differences between the effects produced by increasing the silver concentration and by increasing the pH of the solution, for apart from the increased speed of staining as the pH rises, the pattern of staining alters. As the staining of the section is followed through the pH range after 7 min. impregnation, axonal staining reaches a maximum about pH 8·2–8·4, beyond which further addition of alkali produces no visible effect on the axon. But with increasing pH other structures absorb more silver until the axons become obscured by heavy cellular and background staining. When the impregnation is prolonged for 2½ hr., further changes take place at high pH levels—the whole character of the staining alters, and all the constituents of the section become stained an even reddish brown colour. This change also occurs at intermediate pH levels after 24 hr., but only to a slight extent at the lower end of the pH range. But the changes in staining effects associated with increase in the pH does not occur when the silver concentration alone is increased at pH 6·8. It would appear, therefore, that the effect of increasing concentrations of silver is limited by the pH of the solution and only decreases the time required for the full staining range of a particular pH to be revealed.

When an impregnated section with an adherent film of impregnating solution is placed in a developer, very complex reactions occur. As the developer penetrates the section and mixes with the adherent buffer, the pH fluctuates. These fluctuations affect: (*a*) the tenacity with which the silver is held by the section and thereby indirectly influences the amount of silver removed by the sulphite; and (*b*) the activity of the developing agent, particularly hydroquinone, which is more sensitive to the hydrogen-ion concentration than amidol which is affected only to a slight degree. The lower the pH of the development in hydroquinone sulphite the slower the development, the more silver is removed by the sulphite and less staining occurs, and vice versa. The resultant staining is, therefore, considerably affected by the outcome of the competition between the developer and sulphite, and the complex conditions in which it occurs. This is clearly evidenced by the variations in staining which follow absence of sulphite, or variations in its concentration in the developing solution. It would be incorrect to conclude that the staining depends entirely on the speed of the developing agent and the silver removing properties of the sulphite and the *resultant amount* of silver deposited in the section. For a section treated with sulphite to remove a proportion of the reducible silver before hydroquinone development still stains diffusely, and does not resemble a section developed in a hydroquinone sulphite mixture. Therefore, differential staining appears only as a result of the integrated action of the sulphite and developing agent on the section. But although the *differences* in staining effected by different developers on similar sections can be explained in terms of their different rates and extent of reaction, and on the silver removing properties of the sulphite, it does not explain why a particular developer produces the effect that it does. Why, for example, are amidol sulphite sections diffusely stained, when the same sections developed in hydroquinone sulphite have a differentiated appearance? Apparently whilst the competition between the sulphite and developer is proceeding, some other factor is operating which is partially responsible for the final staining. This aspect of the problem has necessitated a more detailed consideration of the mechanism of silver staining which will be the subject of a future communication.

Holmes (1943) maintained that different developers did not affect the specificity of axonal staining, but that more active developers reducing the silver in the section more completely cause a greater degree of intense staining of all the tissues in the section. As other structures are stained by silver, the term 'specific axon staining' must be qualified, for the phenomenon to which this term really applies is one in which the axons are more intensely stained in contrast to the other tissues, in spite of the silver affinities of the latter. Defined in this way, it is clear from the present experiments that developers do affect the specificity of the stain.

In the last analysis the properties of the fixed tissue determine the amount and distribution of the silver affinities at a particular pH, and the many techniques which have been elaborated were attempts to adjust the silver concentration, pH, temperature and time in the silver bath to the properties of the tissue. But the present experiments indicate that it is incorrect to consider a particular pH and silver concentration as being 'specific' for axonal staining, as the pH, silver concentration, time, and temperature are so inter-related that *within limits* increase or decrease in one has the same effect as decrease or increase in any other. Furthermore, the adjustments of the conditions of impregnation must also ensure that after the certain *time* of reaction between the section and the silver solution demanded by the particular technique, the amount and distribution of the silver in the section is suited to the qualities of the developer employed. So that on development, there results the *same* adequate deposition of silver in the axons which stains them in *contrast to the surrounding tissues.*

SUMMARY

1. Paraffin sections were immersed in buffered silver nitrate solutions of varying pH and silver concentrations for different lengths of time, and developed with various developing agents.

2. The experiments showed an evident inter-relationship between the pH and silver nitrate concentration and the time of impregnation; within limits, increase in one produced effects similar to increase in any of the others.

3. There were, however, important differences between increasing the time, temperature, and silver concentration and increasing the pH.

4. At high pH the sections rapidly assumed an undifferentiated appearance, a change which occurred more slowly as the pH was lowered.

5. To render the axons visible a certain amount of silver must be deposited in them and this amount can be deposited under various conditions of impregnation.

6. Different developing solutions caused profound alteration in the specificity of axonal staining dependent in part on the outcome of the competition between the silver-removing properties of the sulphite and the reduction potential of the developing agent.

7. The effect that a developer could produce was limited by the impregnation.

I would like to express my appreciation of the keen interest shown by Prof. G. A. G. Mitchell in this work, and I am indebted to Dr L. P. Kendal, Lecturer in Physiological Chemistry, and Dr D. C. Henry, Reader in Colloid Physics, for the valuable discussions I have had with them on the various physico-chemical aspects of this problem. My thanks are also due to Mr P. Howarth for the careful preparation of the photomicrographs.

The cost of this research has been defrayed in part by a grant from the Nuffield Foundation.

REFERENCES

Bodian, D. (1936). A new method for staining nerve fibers and nerve endings in mounted paraffin sections. *Anat. Rec.* **65**, 89–97.

Davenport, H. A. & Kline, C. L. (1938). Staining paraffin sections with protargol. 1. Experiments with Bodian's method. 2. Use of *n*-propyl and *n*-butyl alcohol in Hofker's fixative. *Stain Tech.* **13**, 147–160.

Davenport, H. A., McArthur, J. & Bruesch, S. R. (1939). Staining paraffin sections with protargol. 3. The optimum pH for reduction. 4. A two-hour staining method. *Stain Tech.* **14**, 21–26.

Foot, N. C. (1929). Comments on the impregnation of neuroglia with ammoniacal silver salts. *Amer. J. Path.* **5**, 223–238.

Holmes, W. (1943). Silver staining of nerve axons in paraffin sections. *Anat. Rec.* **86**, 157–187.

Kubie, L. S. (1929). Staining of tissues of the central nervous system with silver. The influence of the strength of the reducing agent. *Arch. Neurol. Psychiat., Chicago*, **22**, 135–138.

Kubie, L. S. & Davidson, D. (1928). The ammoniacal silver solutions used in neuropathology. Their staining properties, chemistry, and methods of preparation. *Arch. Neurol. Psychiat., Chicago*, **19**, 888–903.

Pearson, A. A. & O'Neill, S. L. (1946). A silver-gelatin method for staining nerve fibers. *Anat. Rec.* **95**, 297–301.

Romanes, G. J. (1950). The staining of nerve fibres in paraffin sections with silver. *J. Anat., Lond.*, **84**, 104–115.

Silver, M. L. (1942). Colloidal factors controlling silver staining. *Anat. Rec.* **82**, 507–529.

EXPLANATION OF PLATES

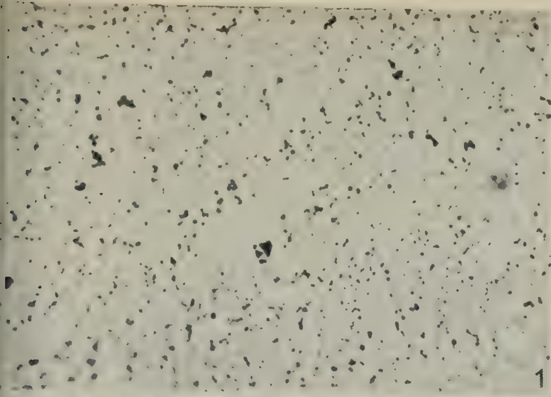
All the photomicrographs have been taken under standard conditions of light intensity, exposure time, development and printing. All the sections are of the sympathetic ganglion of the rabbit fixed in the solution of Davenport & Kline (1938).

Plate 1

Fig. 1. The section impregnated at pH 6·8 for 7 min. is unstained. × 520.

Fig. 2. The section impregnated at pH 8·7 shows clearly differentiated axons and cells. × 520.

Fig. 3. In the section impregnated at pH 9·1 for 7 min., heavy diffuse staining obscures axonal detail. × 520.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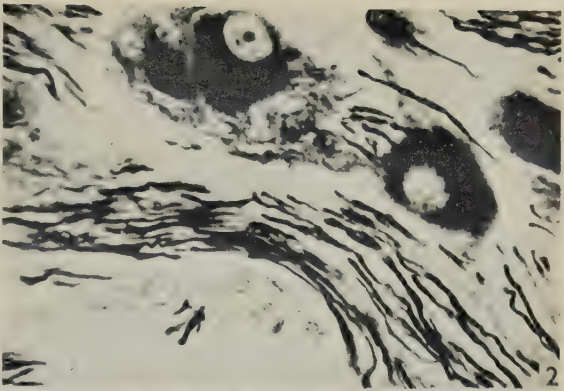
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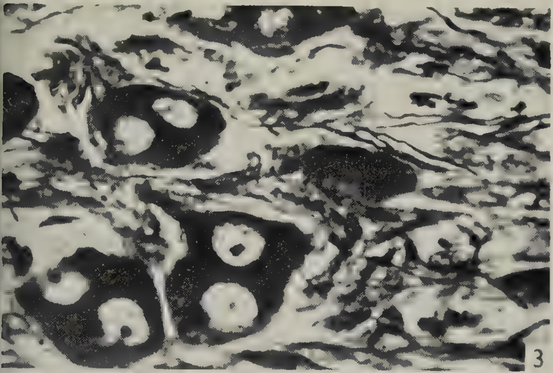
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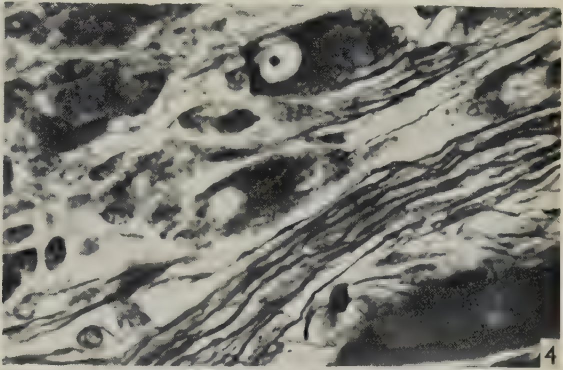
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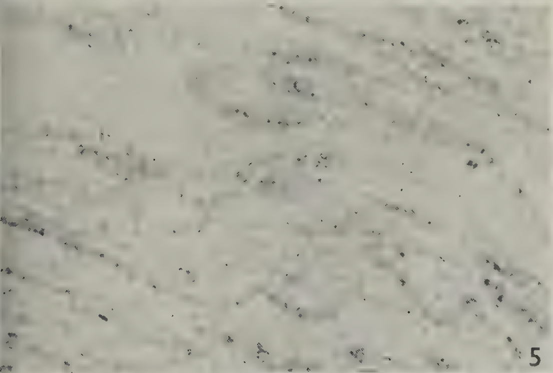
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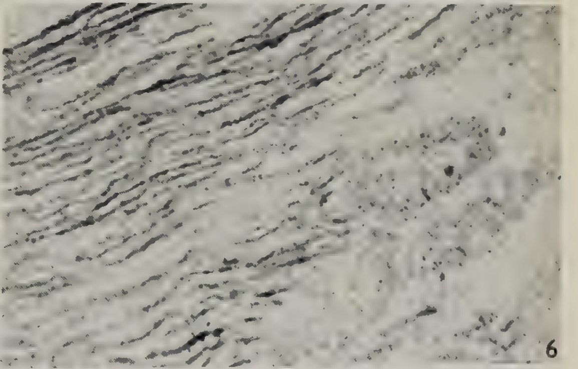
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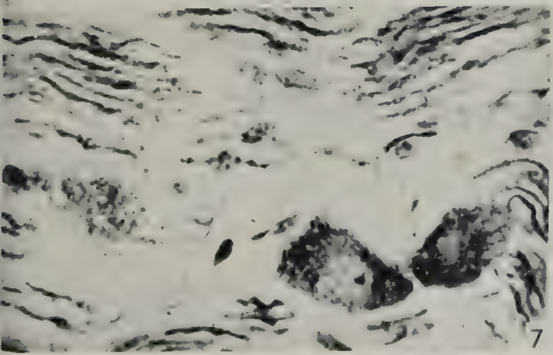
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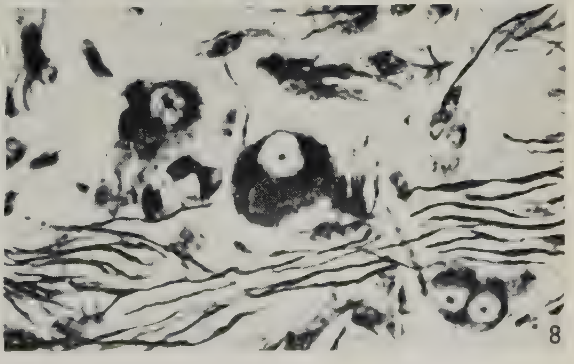
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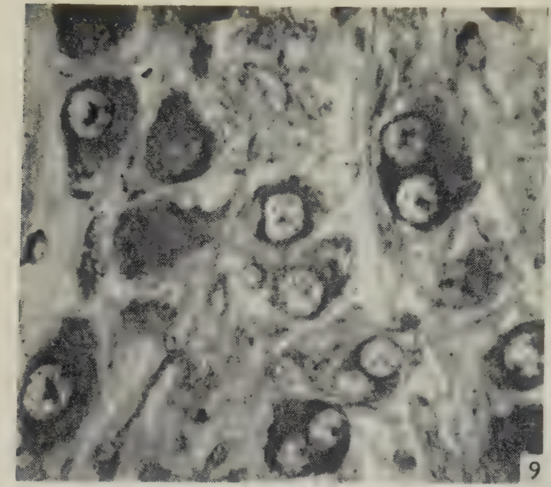
SAMUEL—Impregnation and development in silver staining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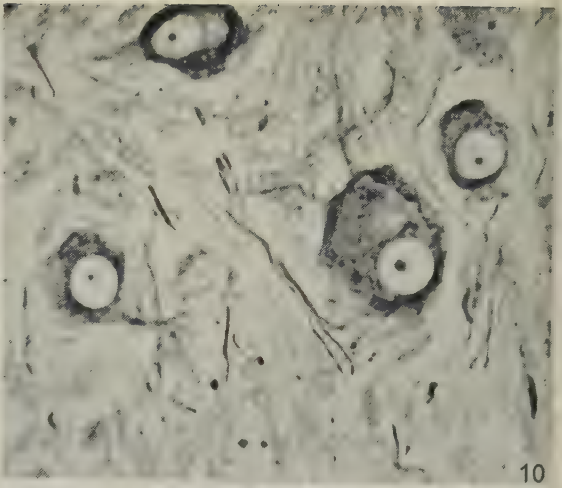
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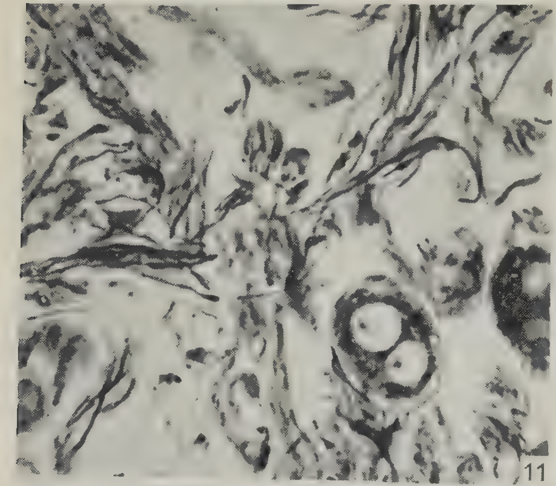
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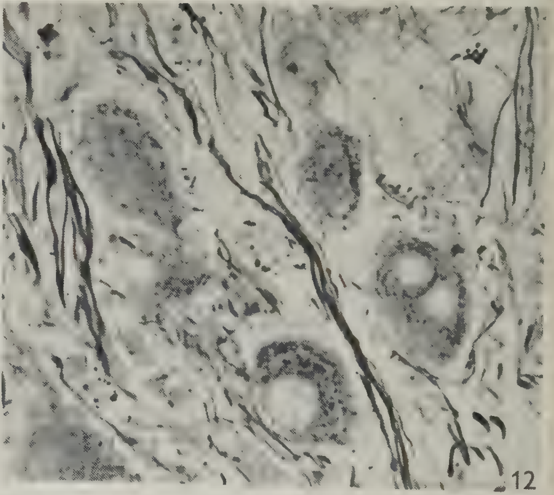
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SAMUEL—Impregnation and development in silver staining

Fig. 4. A section impregnated at pH 6·8 for 2½ hr. showing the effect of prolonged incubation at this pH, compare with fig. 1. × 520.

Fig. 5. A section impregnated at pH 6·8 for 45 min. × 520.

Fig. 6. A section impregnated at pH 6·8 for 1 hr. 15 min. × 520.

Fig. 7. A section impregnated at pH 6·8 for 1 hr. 45 min. Figs. 5, 6, 7 and 4 illustrate how axonal staining is a gradual process and is not an end-point phenomenon. × 520.

Fig. 8. A section impregnated at pH 6·8 for 15 min. with 10 c.c. of silver nitrate per 100 c.c. of buffer solution. Compare with fig. 1 which resembles a section impregnated for 15 min. when 1 c.c. of 1 % silver nitrate was added to the buffer. × 520.

PLATE 2

Fig. 9. Section impregnated at pH 7·4 for 1 hr., developed in 1 % amidol in 10 % sodium sulphite. The axons are barely visible in the diffusely stained background. Note the intense staining of the cells. × 520.

Fig. 10. A similar section to that shown in fig. 9 but developed in a 1 % hydroquinone in 10% sodium sulphite. The delicately stained axons are readily seen in the clear background. Note the uneven stain of the cell cytoplasm and lack of nuclear detail as compared with fig. 9. × 520.

Fig. 11. A section of sympathetic ganglion of rabbit impregnated at pH 8·5 for 1 hr. and developed in a 1 % hydroquinone in 10 % sodium sulphite. × 520.

Fig. 12. A section similar to that shown in fig. 11 but treated for 15 sec. in a 2·5 % solution of sodium sulphite before development. There is much less staining in the cells and background but the axons are hardly affected. × 520.

Fig. 13. When the time in the 2·5 % sodium sulphite is prolonged for 2 min. there is a complete absence of staining. × 520.

THE MECHANISM OF SILVER STAINING

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Though much research has been devoted to the elaboration of silver stains for nervous tissue, there have been few experimental investigations of the mechanism of the staining process. Liesegang (1911) claimed that during the impregnation in the silver bath, ultramicroscopic particles of metallic silver were deposited in the sections as silver 'nuclei', and it was on these 'nuclei' that further silver deposition occurred during development. Thus the final staining of the tissue was determined by the initial distribution of the silver 'nuclei'. This theory was generally accepted until Owens & Bensley (1929) and Nageotte & Guyon (1930) suggested that the staining was more dependent on the physical properties of the tissue. Zon (1936) concluded that the differences in stainability of the tissue elements were due solely to the differences in the protective properties of their colloids, which permitted varying degrees of precipitation of silver within them. Silver (1942) thought that during the development phase of silver staining negatively charged silver micelles were formed and these were precipitated by the positive charges carried by the tissues. The pH of the silver solution was important only in so far as it affected the charge on the micelle, rendering it more or less liable to flocculation, but the pH of the developing solution was of extreme importance in so far as it affected the charge on the tissues and hence the distribution of the silver.

As none of the previous opinions seemed to be based on incontestable evidence, it was considered that a further investigation into the mechanism of silver staining might throw further light on the problem.

MATERIALS AND METHODS

The experiments to be described below were carried out on 10 μ paraffin sections of the sympathetic ganglia and spinal cords of rabbits fixed in a modified Hofker's solution (Davenport & Kline, 1938). The sections were impregnated in a silver borate solution (for details see previous communication) and subjected to various experimental procedures.

EXPERIMENTS

Silver's (1942) *hypothesis*

I have already shown, in experiments described previously, that different developers produce different staining effects, and that the impregnation process exercises a controlling influence on the staining caused by a particular developer. But it was necessary to discover whether, as Silver (1942) believed, the staining was entirely dependent on the physical state of tissue colloids during the development phase, because, if this was the case, variously impregnated sections, after washing and drying, should be in a uniform physical state; the staining on development should then depend solely on the state of the section in the developer *as determined by the properties of the developing solution*, and the staining of the sections should always be the same, irrespective of the conditions of the impregnation.

However, no significant difference was found between the sections impregnated at the same pH, whether they had been dried before development or not. Thus sections impregnated at pH 7·4 and 8·8 and washed and dried (Pl. 1, figs. 1, 3) resembled sections impregnated at pH 7·4 and 8·8 which had not been dried (Pl. 1, figs. 2, 4). There were, however, marked differences between the dried sections —the staining was much less complete at pH 7·4 (Pl. 1, fig. 1) than at pH 8·8 (Pl. 1, fig. 3).

Liesegang's (1911) *hypothesis*

Was it possible, as Liesegang (1911) postulated, that reducible silver was thrown down by the developer on silver 'nuclei' of reduced metallic silver, deposited in the section during the impregnation? Certainly sections were capable of reducing silver, for after prolonged incubation, particularly at high pH levels, silver was deposited in the section and the tissue elements were visible without development or toning. It was found that this silver was not removed by sodium sulphite.* If this type of silver was deposited in sections in short impregnations it was likely that this also would not be removed by sodium sulphite. But sections treated by sulphite after short impregnations were quite unstained and silver 'nuclei', if present, were invisible and could not be revealed by gold toning. Attempts were made to render them visible by a process of physical development, a technique used in miniature photography, in which the latent image is developed by depositing additional silver on it from a silver containing developer solution. The developer finally adopted was that used in the Pearson & O'Neill (1946) silver method (40 c.c. 3 % gelatin, 20 c.c. 2 % silver nitrate and 10 c.c. 1 % hydroquinone), a solution differing little from that of Liesegang (1911) who used gum arabic instead of gelatin. This provided a source of silver particles to deposit on any silver 'nuclei' present, without gross precipitation on the slide.

The routine adopted was:

(1) Impregnate in buffered silver nitrate solution.
(2) Rinse in three changes of distilled water.
(3) Remove the reducible silver by treatment with 2·5 % sulphite for 5 min.
(4) Wash in running tap water for 10 min. and rinse in distilled water.
(5) Place in Pearson's developer for 3 min. at 28° C. in a Coplin jar in a thermostatically controlled water-bath.

No toning was employed.

This method of processing will hereafter be referred to as physical development. It differed basically from that of Liesegang (1911), because he failed to remove the reducible silver from the sections prior to their treatment in his gum arabic-silver hydroquinone mixture.

The present method demonstrated that the impregnation affected the section, as staining occurred when the section was placed in the physical developer. Control sections subjected to identical treatment, but without the addition of silver to the second buffer solution, did not stain in any way. It was concluded that a *silver-section* reaction occurring during the impregnation resulted in the deposition of silver in the section. This determined the distribution and amount of further silver derived from the physical developer. The precise form of the silver deposited in the section during impregnation could only be surmised, but (*a*) it was ultramicroscopic, (*b*) it caused autocatalysis in a hydroquinone, gelatin, silver nitrate solution (see below), and (*c*) since reducing groups exist in the tissues (Liang, 1947) it was surmised that the silver was in the form of ultramicroscopic particles of reduced silver, the so-called silver 'nuclei'. By removing the reducible silver, physical development could be carried out completely independently of the conditions of impregnation, and because the conditions of physical development were constant any variation in the staining could be attributed solely to altered impregnation. It proved possible by this method to study the disposition and amount of the silver 'nuclei' under different conditions.

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* Earlier experiments had shown that sections treated with sodium sulphite before development did not stain, i.e. the sulphite removed the reducible silver from the section.

To demonstrate the reducible silver in the section under varying conditions, the sections were quickly rinsed in distilled water and then placed in a rapid developer, 1 % hydroquinone solution; the rinse served to remove any adherent buffer silver solution carried over mechanically from the impregnating bath which might affect the staining.

As a 1 % hydroquinone in a 10 % sodium sulphite solution had been found to produce well-differentiated staining, sections were also developed in this solution for comparison with the physically developed sections.

Sections were impregnated in a solution containing 1 c.c. of 1 % silver nitrate buffered over a pH range 6·8–9·1 for periods of 15 min., 2½ and 24 hr. They were then:

(1) treated with 2·5 % sodium sulphite and physically developed;
or (2) rinsed in distilled water and developed in a 1 % hydroquinone solution;
or (3) rinsed in distilled water and developed in a 1 % hydroquinone in a 10 % sodium sulphite solution.

The sections were not toned in these experiments.

Physical development, silver 'nuclei'

Fifteen min. impregnation. At pH 6·8 the axons and cells at the periphery of the section were stained varying shades of brown. Over the intermediate pH range 8–8·4 the staining became more even, but thereafter the staining decreased until at pH 9·1 (Pl. 1, fig. 5) only faint axonal staining could be seen—the cells and their nuclei stained at the periphery, with more nuclei staining towards the centre.

Two and a half hours' impregnation. At all pH levels the whole section was stained. In the sections impregnated at pH 6·8 (Pl. 1, fig. 6) the cells were dark grey green in colour, their nuclei were deep brown, and the axons were clearly stained brown and black in a clear background. As the pH rose the staining diminished, until at pH 9·1 (Pl. 1, fig. 7) the neuronal cytoplasm was feebly stained, the nuclei were brown, and the axons were almost invisible in the stained background tissue, but reticulin staining was clearly evident in arterial walls and in the capsule of the ganglion.

Twenty-four hours' impregnation. After prolonged incubation, the sections became less heavily stained on physical development and showed uniform brown staining, particularly in high pH impregnations.

Development in 1 % hydroquinone. Reducible silver

Fifteen min. impregnation. At pH 6·8 only a faint precipitate covered the section. With rising pH the amount of reducible silver greatly increased and in sections impregnated at pH 9·1 (Pl. 1, fig. 8) considerable staining of the cells, nuclei and background occurred.

Two and a half hours' impregnation. At each pH the reducible silver greatly increased as the impregnation was prolonged. Apart from the great overall increase in reducible silver, there was a difference too in its distribution. As the pH rose from 6·8 (Pl. 1, fig. 9), the staining of the background increased in intensity and the neurons changed from pale yellow through deepening shades of brown until they were almost black at pH 9·1 (Pl. 2, fig. 10). The axons, however, increased in staining from pale yellow at pH 6·8 to brown up to pH 8·2–8·4, but then the staining diminished and became scarcely visible at the high pH levels, partly because of the heavy granular staining of the background. At all pH levels, the sections developed in 1 % hydroquinone had a coarse granular appearance.

Twenty-four hours' impregnation. After prolonged incubation, there was little difference in the staining at the lower end of the pH range. As the pH increased the sections became increasingly brown before development, and consequently it was impossible to determine how much of the staining could be attributed to the reducible silver. But two changes were observed in the developed sections: (*a*) they were more evenly stained than after 2½ hr., and (*b*) they were less granular in appearance.

Hydroquinone sulphite development

Fifteen min. impregnation. Sections impregnated at pH 6·8 were unstained; staining did not appear until the pH rose above a value of 8. Thereafter the axons and cells and cell nuclei gradually increased in density until at pH 9·1 they were heavily stained (Pl. 2, fig. 11).

Two and a half hours' impregnation. After 2½ hr. the cells and axons were faintly stained at pH 6·8 (Pl. 2, fig. 12) and the sections impregnated at the intermediate pH levels also showed some increase in staining. At the highest pH levels there was *less* staining than before, and the distribution of the stain had altered. At pH 9·1, the sections were stained a diffuse even brown in colour and the axons were scarcely visible in background tissue (Pl. 2, fig. 13), cf. pH 9·1 at 15 min. (Pl. 2, fig. 11).

Twenty-four hours' impregnation. Prolonged incubation caused little change at low pH, but the sections became increasingly and uniformly brown in colour after development, as the pH rose.

DISCUSSION

Before considering the significance of the above experiments, it is necessary to relate briefly certain relevant information.

James (1939*a*, *b*; 1940) has shown that the reduction of silver nitrate in a gelatin solution by hydroquinone is:

(1) Catalysed by the addition of colloidal silver, and the colloidal silver initiates

an autocatalytic process in which the silver is adsorbed on to the metallic silver particles and reduced *in situ.*

(2) The rate of reduction is increased by increasing concentrations of silver ions up to a point beyond which further increase has no effect.

(3) The oxidation products of hydroquinone accelerate its action.

(4) In the gelatin silver nitrate hydroquinone, new particle formation does not occur for 16 min.

Though the conditions in my experiments are not identical with those described by James, it is reasonable to suppose that the staining which occurs when a section is placed in the physical developer is due to the catalytic action of the silver deposited in the section during the impregnation, particularly as control sections do not stain on physical development. The amount and distribution of the silver passing into the section in physical development is determined by: (*a*) the amount and distribution of the silver 'nuclei', and (*b*) the amount of physical development.

Sheppard (1944) mentioned several facts which are relevant to the present discussion:

(1) The deposition and development of silver ions on metallic silver 'nuclei' probably assumes a filamentous form, which becomes disrupted because of the thermal energy of the system.

(2) Silver ions react with sulphite

$$Ag^+ + SO_3 = AgSO_3$$

and form perhaps even higher complexes. The silver ions in this way are more protected from the developer and the silver catalytic reaction is retarded.

(3) The presence of sulphite decreases the tendency for clumping of metallic silver particles.

In addition, it has been shown in the present research that: (*a*) the sulphite also removes reducible silver from the section, and (*b*) during impregnation, two essential fractions of silver are deposited in the section: (*a*) a fraction which is reduced by the developer, 'reducible silver'; (*b*) a fraction of silver which is not affected by sulphite and which is probably in the form of minute silver particles or 'nuclei'.

The colour of silver sols. varies from yellow to brown to red as the particle size increases. If, however, a section impregnated at pH 8·5 is subjected to physical development for increasing lengths of time up to 8–10 min., the colour changes in the section vary from yellow to deep brown to black, indicating that the progressive deposition of silver which occurs does not result in a uniform growth of particle size. This is probably due to the nature of the medium in which it occurs and the mechanism of growth of the initial 'nuclei'.

Bearing the above facts in mind and correlating them with the experimental findings, it is possible to postulate the following explanations of the staining effects produced on physical development, and on development in the hydroquinone and hydroquinone sulphite solutions.

Physical development

A section removed from the impregnating solution and rinsed and treated with sodium sulphite solution contains a number of ultramicroscopic metallic silver 'nuclei'. At pH 6·8, the axons and cells contain a large number of 'nuclei' after 2½ hr. impregnation; thus, when placed in the physical developer, vigorous catalysis of silver occurs, the silver ion coming in contact with the silver 'nuclei' and being reduced *in situ*. This freshly deposited metallic silver in turn becomes a silver ion receiving surface, and by this process of autocatalysis, rapid deposition of silver occurs in the axons and cells, which in 3 min. become black and grey greenish brown in colour respectively (Pl. 1, fig. 6). As the pH rises the amount of silver 'nuclei' per unit volume of tissue decreases, and less staining occurs under the same conditions of physical development, until at pH 9·5 with 15 min. impregnation only faint axonal staining (Pl. 1, fig. 5) is seen, the neurons having a pale cytoplasm and black nuclei; at 2 hr. 30 min. axonal staining has all but disappeared, though the section is now more evenly stained, showing pale yellow cells, brown nuclei, and extensive reticular staining (Pl. 1, fig. 7).

Development in the hydroquinone and hydroquinone sulphite solutions

As the pH rises the amount of reducible silver in the section rises but its final distribution, and hence the staining, varies with the developer used.

In the hydroquinone sulphite developer, development takes place more slowly than in hydroquinone, as the oxidation products combine with the sulphite, and as the sulphite forms more stable complexes with the silver it removes from the section. The formation of silver sulphite complexes simulates the condition in the physical developer, i.e. there is formed a solution of reducible silver in the developing agent. Development again commences on the silver 'nuclei' in the cells and axons, and proceeds at increasing speed as the silver available for catalysis increases in amount. Meanwhile, the sulphite removes silver from the section where development proceeds least rapidly. This silver partially diffuses away from the section, but is partially reduced and deposited on the increasing surface of metallic silver. In effect, therefore, a re-distribution of the reducible silver takes place in the section. Thus, in the hydroquinone sulphite, as the reducible silver increases with rising pH, its deposition is still maximal where the development commences; and where the silver 'nuclei' are present in greatest concentration, increasing amounts of silver are deposited in the cells and axons. Therefore, although at pH 9·1, with 15 min. impregnation, there are very few 'nuclei' in the axons and cells (Pl. 1, fig. 5), development commences on them. It then progresses at increasing speed with the deposition of the large amounts of silver removed from the adjoining regions providing additional catalytic silver, and intense staining results (Pl. 2, fig. 11). (This cannot occur in the rapid hydroquinone developer which does not contain sulphite and which reduces the silver *in situ*, cf. Pl. 1, fig. 8; Pl. 2, figs. 10, 11). But when the impregnation is prolonged for 2½ hr. at pH 9·1, and the section is developed in hydroquinone sulphite solution, the cells become deep brown in colour and the axons are barely visible in the brown background tissue—this is seen in Pl. 2, fig. 13. As the same developer is used, the change must be due to alteration in the properties of the section as a result of the increased incubation. Reducible silver is still present in large amounts (Pl. 2, fig. 10), and it is concluded that the change probably affects the silver 'nuclei'.

That change can occur in the 'nuclei' is evidenced by the changes in the response to physical development of sections impregnated at pH 8·5 for 1 hr. and 24 hr. In the former (Pl. 2, fig. 14) all the structures are jet black; in the latter much less silver is deposited in the section (Pl. 2, fig. 15) indicating a diminution in the active catalytic silver. This changing response to physical development is slightly evident at lower pH values, but is more obvious, and occurs with increasing rapidity, as the pH of the impregnating solutions rises, until at pH 9·1 it appears within 2½ hr. Possibly in the course of the impregnation the larger 'nuclei' grow at the expense of the smaller ones, so reducing the catalytic surface of the silver 'nuclei'. Some slight evidence in favour of the conception of growth of particle size is that sections impregnated at pH 9·1 for 15 min. and physically developed, when viewed by dark ground illumination, show marked light diffraction in their cell nuclei (Pl. 2, fig. 16). Two hours fifteen minutes later this diffraction has almost disappeared (Pl. 2, fig. 17). This may perhaps indicate that in 15 min. a multifocal deposition of silver occurs on physical development, giving a greater irregularity of surface than when less silver deposition occurs more slowly in relationship to the fewer larger silver 'nuclei' present after 2½ hr. impregnation.

Thus in the sections impregnated at pH 9·1 for 2½ hr. this decrease in the rate of development in the hydroquinone sulphite developer allows greater diffusion of the sulphite-removed silver from the section, and results in less silver being deposited in the cells and axons (Pl. 2, fig. 13), but more in the background tissues due to increased amounts of silver 'nuclei' there. The decrease in catalytic surface has less effect on the more rapid hydroquinone developer and heavy deposition of silver still occurs (Pl. 2, fig. 10). With further prolongation of the development the hydroquinone sections are also stained a paler brown colour.

Do the charges on the colloids of the section play any part in the staining reaction? Silver (1942) believed that the reducible silver, under the influence of the developer, produced negatively charged silver micelles which are distributed and deposited according to the electrical charges of the tissues. The charges of the tissues are determined by their chemical environment. If this is true, sections impregnated at different pH levels and rinsed and dried in an incubator before development should exhibit staining determined only by the properties and pH of the developer. This is not so. Sections treated in this manner stain exactly the same as sections placed directly in the developer from the impregnating bath, i.e. the impregnation plays a decisive role. As the staining process takes place in the colloidal media of the tissue elements the properties of the colloids must play an important part in staining. The chemical and physical properties of the colloid of the section, however, have complex interrelationships and it is as yet impossible to say exactly how these interrelated properties, which change as the reaction proceeds, affect the silver staining.

The experiments indicate that when responding to developer and reducible silver, silver 'nuclei' exert a determining influence. Liesegang (1911) claimed that the silver 'nuclei' serve as centres on which the reducible silver is mechanically deposited on development. In his experiments he omitted to remove the reducible silver in the section, which would therefore be reduced by the hydroquinone in his developer solution. Thus his results do not demonstrate the true sites of deposition of the 'nuclei' as determined by the impregnation. Furthermore, his conception does not explain why after 15 min. impregnation at pH 9·1 (Pl. 2, fig. 11) intense axonal staining occurs when very few 'nuclei' are present (Pl. 1, fig. 5). One would expect at least a degree of 'nuclear' deposition comparable with that produced at pH 6·8 after 2½ hr. impregnation (Pl. 1, fig. 6), if there is any quantitative correlation between staining and the 'nuclei'. In fact, in no case does the appearance of the untoned sections after hydroquinone development correspond with the distribution of silver 'nuclei' as seen by physical development, and there is no basis to explain why different developers, contrary to the opinion of Holmes (1943), produce such different effects. Nor does Liesegang's hypothesis explain why the staining should alter so profoundly at pH 9·1 between 15 min. (Pl. 2, fig. 11) and 2½ hr. (Pl. 2, fig. 13) though the identical developer is used. Zon (1936) recognized the presence of reduced silver in the tissue, but considered that it is so protected by the colloid in which it is embedded that it exerts no significant effect on staining. This view is not supported by the changing effects occurring on physical development when the conditions of impregnation with buffered silver nitrate are altered.

To summarize, it may be said that the changing distribution of silver 'nuclei' as seen by physical development, after varying impregnation conditions, can be related to the differences in staining produced by different developers. The present research seems to indicate that the silver 'nuclei' play a dynamic part in a reaction in which the properties of the developer, and the amount and distribution of reducible silver, also have their own decisive influence.

SUMMARY

1. When immersed in a buffered silver solution two types of silver are deposited in the section: (*a*) a fraction of silver reduced by the section probably as minute metallic silver particles or 'nuclei'; (*b*) a fraction of silver which is reduced by the developer, reducible silver.

2. The amount and distribution of the silver 'nuclei' and reducible silver depends upon: (*a*) the properties of the section; (*b*) the pH of the solution; (*c*) the silver concentration; and (*d*) the time and temperature of incubation.

3. The silver 'nuclei' play a dynamic role as catalytic centres for silver reduction.

4. There is a complex interrelationship between these centres, the reducible silver, the developer, and the tissues. All play fundamental roles in the final staining of the section.

I should like to express my appreciation of the keen interest shown by Prof. G. A. G. Mitchell in this work, and I am indebted to Dr L. P. Kendal, Lecturer in Physiological Chemistry, and Dr D. C. Henry, Reader in Colloid Physics, for the valuable discussions I have had with them on the various physico-chemical aspects of this problem. My thanks are also due to Mr P. Howarth for the careful preparation of the photomicrographs.

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REFERENCES

Davenport, H. A. & Kline, C. L. (1938). Staining paraffin sections with protargol. 1. Experiments with Bodian's method. 2. Use of *n*-propyl alcohol and *n*-butyl alcohol in Hofker's fixative. *Stain Tech.* **13**, 147–160.

Holmes, W. (1943). Silver staining of nerve axons in paraffin sections. *Anat. Rec.* **86**, 157–187.

James, T. H. (1939*a*). The reduction of silver ions by hydroquinone. *J. Amer. Chem. Soc.* **61**, 648–652.

James, T. H. (1939*b*). Mechanism of photographic development. 1. The effect of oxidation products on the development process and the nature of the induction period. *J. phys. Chem.* **43**, 701–719.

James, T. H. (1940). Mechanism of photographic development. 11. Development by hydroquinone. *J. phys. Chem.* **44**, 42–57.

Liang, H. M. (1947). A new method for staining nerves and their endings using the Schiff reaction. *Anat. Rec.* **99**, 511–521.

Liesegang, R. E. (1911). Die Kolloidchemie der histologischen Silberfärbungen. *Kolloidchem. Beih.* **3**, 1–46.

Nageotte, J. & Guyon, L. (1930). Reticulin. *Amer. J. Path.* **6**, 631–653.

Owens, H. B. & Bensley, R. R. (1929). On osmic acid as a micro-chemical reagent, with special reference to the reticular apparatus of Golgi. *Amer. J. Anat.* **44**, 79–109.

Pearson, A. A. & O'Neill, S. L. (1946). A silver gelatin method for staining nerve fibers. *Anat. Rec.* **95**, 297–301.

Sheppard, S. E. (1944). Colloidal chemical aspects of photographic development. In *Colloid Chemistry. Theoretical and Applied*, **5**, 472–512, edited by Jerome Alexander. New York: Reinhold Publishing Corporation.

Silver, M. L. (1942). Colloidal factors controlling silver staining. *Anat. Rec.* **82**, 507–529.

Zon, L. (1936). The physical chemistry of silver staining. *Stain Tech.* **11**, 53–67.

EXPLANATION OF PLATES

All the photomicrographs have been prepared under standard conditions of light intensity, exposure time, development and printing.

Plate 1

Fig. 1. Transverse section spinal cord of rabbit. After impregnation at pH 7·4 the section was washed and dried before development. Note the similarity to fig. 2 and the difference from fig. 3. ×105.

Fig. 2. Section treated as in fig. 1 but not dried before development. ×105.

Fig. 3. Transverse section spinal cord of rabbit. After impregnation at pH 8·8 the section was washed and dried before development. Note the similarity to fig. 4. ×105.

Fig. 4. Section treated as in fig. 3 but not dried before development. ×105.

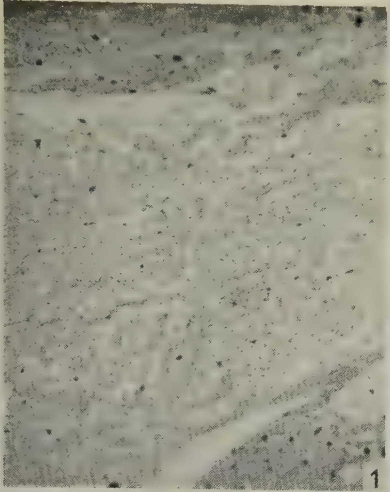
Fig. 5. Section of sympathetic ganglion of rabbit. Physical development after impregnation at pH 9·1 for 15 min. indicates that few silver 'nuclei' are present in the tissues. ×520.

Fig. 6. Section of sympathetic ganglion of rabbit. Physical development after impregnation at pH 6·8 for 2½ hr. The axons, the cells and their nuclei are well stained, compare with fig. 7. ×520.

Fig. 7. A section treated as in fig. 6 but impregnated at pH 9·1. The section is feebly stained, and no axonal staining can be seen. ×520.

Fig. 8. Section of sympathetic ganglion of rabbit impregnated at pH 9·1 for 15 min. and developed in 1% hydroquinone. After 15 min. there is a considerable amount of reducible silver in the section, compare with fig. 9. ×520.

Fig. 9. Section of sympathetic ganglion of rabbit impregnated at pH 6·8 for 2½ hr. and developed in 1% hydroquinone. Only a small amount of reducible silver is present in the section. ×520.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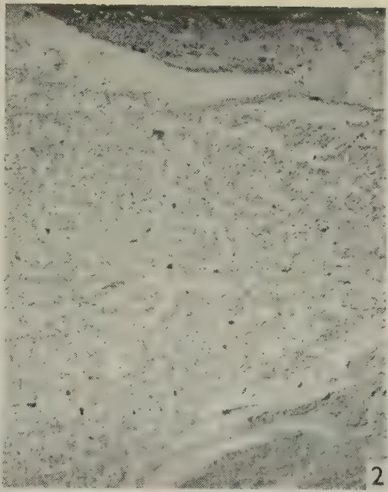
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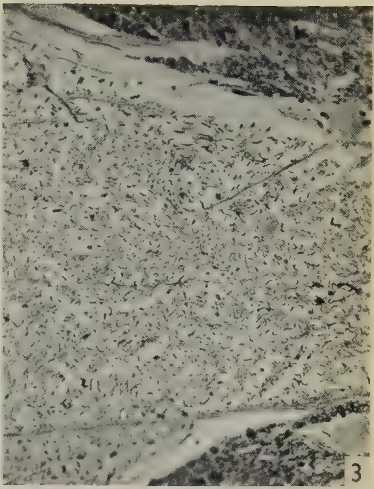
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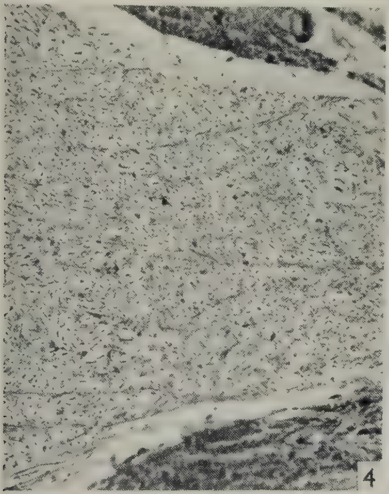
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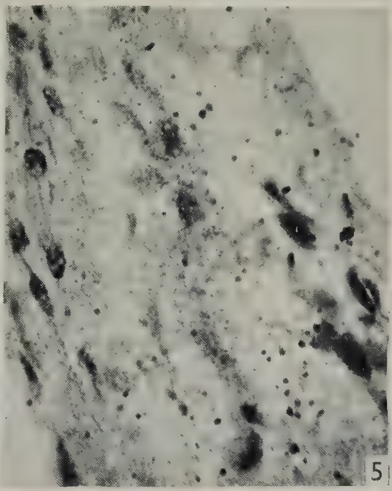
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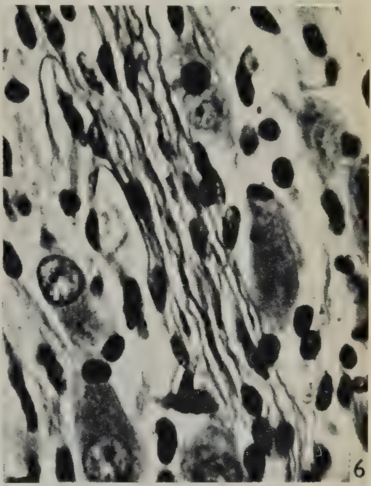
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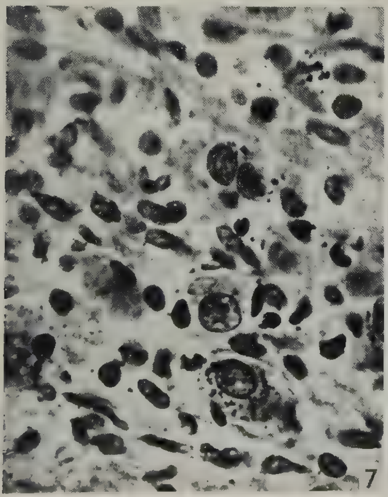
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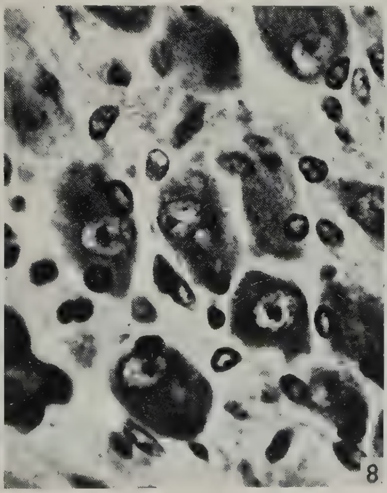
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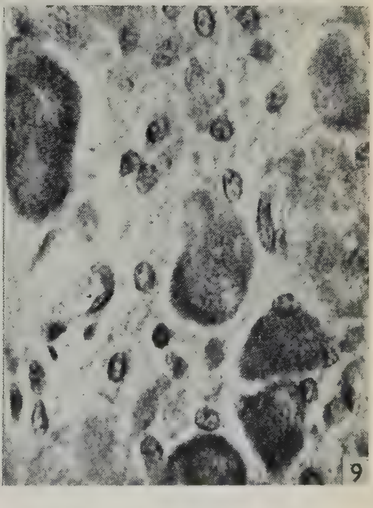
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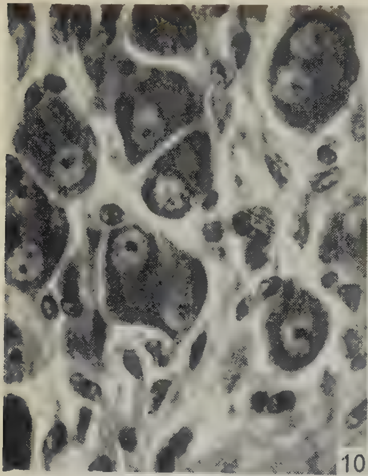
SAMUEL—THE MECHANISM OF SILVER STAINING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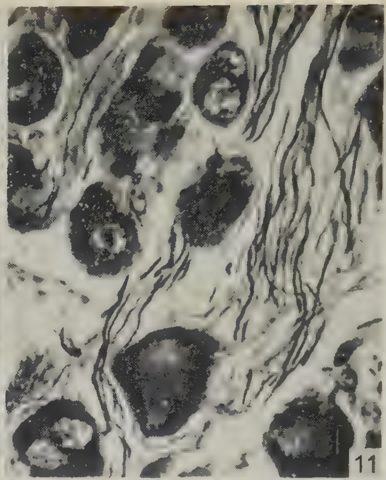
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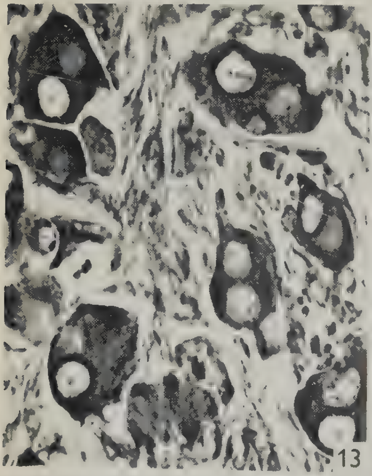
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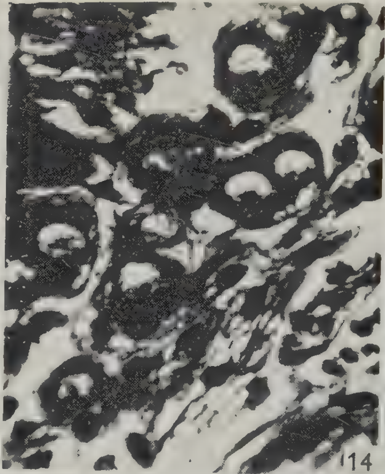
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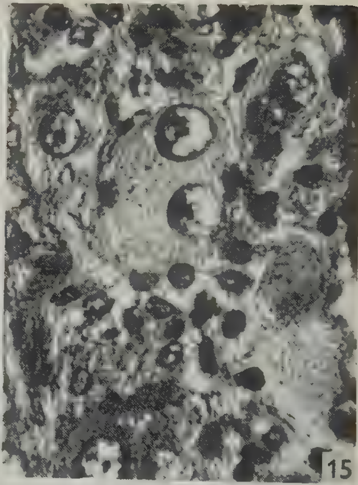
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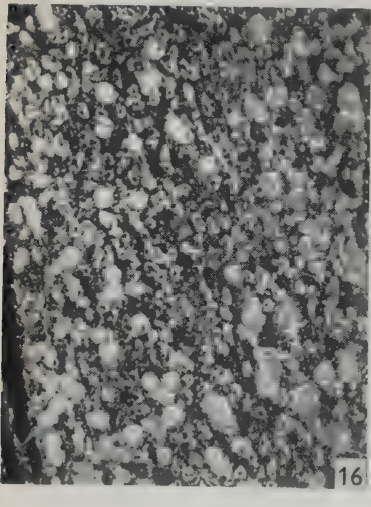
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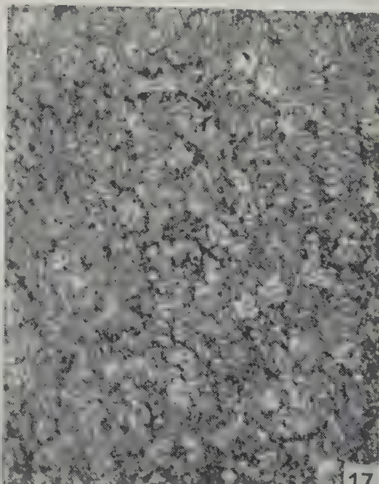
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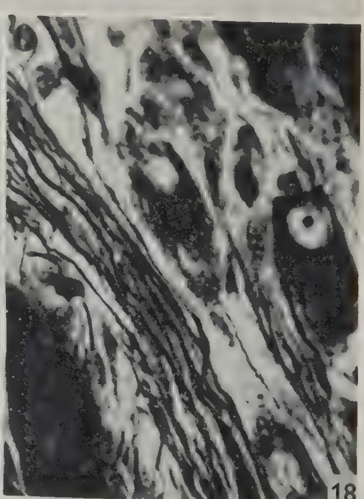
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SAMUEL—The mechanism of silver staining

PLATE 2

Fig. 10. A section treated as in fig. 9 but impregnated at pH 9·1. The heavy diffuse staining indicates how the reducible silver increases as the pH rises, compare with fig. 9, and as the time of impregnation is prolonged, compare with fig. 8. × 520.

Fig. 11. Section of sympathetic ganglion of rabbit impregnated at pH 9·1 for 15 min. and developed in 1 % hydroquinone in 10 % sodium sulphite. Deeply stained axons and neurons may be seen. This section has *not* been toned. × 520.

Fig. 12. Section of sympathetic ganglion of rabbit impregnated at pH 6·8 for 2½ hr. and developed in 1 % hydroquinone in 10 % sodium sulphite. Untoned. The section is faintly stained but similar sections when toned, see fig. 18, resemble fig. 6. × 520.

Fig. 13. A section treated as in fig. 12 but impregnated at pH 9·1. The diffuse staining should be compared with fig. 11 which was impregnated for 15 min. only. × 520.

Fig. 14. A section of sympathetic ganglion of rabbit impregnated at pH 8·5 for 1 hr. and physically developed for 10 min. at 53·5° C. The whole section is jet black in colour, compare with fig. 15. × 520.

Fig. 15. A section treated as in fig. 14 but impregnated for 24 hr. After the prolonged incubation there is much less staining on physical development. × 520.

Fig. 16. A section of sympathetic ganglion of rabbit impregnated at pH 9·1 and physically developed for 15 min. photographed with dark ground illumination. Note the highly refractile cell nuclei. × 220.

Fig. 17. A section treated as in fig. 16 but impregnated for 2½ hr. The cell nuclei are no longer refractile. × 220.

Fig. 18. A section of sympathetic ganglion of rabbit impregnated at pH 6·8 for 2½ hr., developed in 1 % hydroquinone in 10 % sodium sulphite and toned. When toning is used the close relationship between the distribution of the silver 'nuclei' and the staining effect produced by the hydroquinone sulphite becomes clearly apparent, cf. figs. 6, 12 and 18.

OBSERVATIONS ON THE OLFACTORY APPARATUS AND THE TELENCEPHALON OF *ANOLIS*, A MICROSMATIC LIZARD*

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Most reptiles have a well-developed olfactory and vomero-nasal apparatus; in only one order, the Crocodilia, is there a complete absence of the vomero-nasal component. Some of the Lacertilia, however, have been described as 'microsmatic', including the anoles which are a group of essentially arboreal New World lizards.

The diencephalon and midbrain of *Anolis carolinensis* were described in detail by Huber & Crosby (1933) but, so far as we are aware, there is no comprehensive description of the telencephalon. Occasional references to certain features of this part of the brain were made by Kappers, Huber & Crosby (1936), including a statement that the olfactory bulbs 'are small masses, conical in outline and situated at the base of the hemisphere, overlapped by its frontal pole'. Crosby & Humphrey (1939*a*, *b*) placed *Anolis* with certain other iguanids in a group of 'lizards with no accessory olfactory bulbs'; while in a more recent publication by Pratt (1948) it is held that the epithelium of the vomero-nasal organ in *A. alligator* is 'completely non-sensory', and that of the nasal sac 'almost non-sensory' in nature.

Several accounts of the normal anatomy of the typical lacertilian forebrain are already available, and in spite of differences in the species studied, of the various techniques adopted and of conflicting terminologies and interpretations, there emerges a remarkably constant pattern of forebrain morphology. However, since olfactory connexions have been thought to play an important part in determining this morphology, it was decided to investigate the condition of the telencephalon and olfactory organs in specimens of *Anolis* which recently became available. As will be seen, some of our findings differ fundamentally from those of the earlier writers.

MATERIAL AND METHODS

Specimens of three species of *Anolis* were available, *A. lineatopus*, *garmani* and *grahami*. *A. garmani* is considerably larger than either of the other two species, but no other difference relevant to the present investigation was found between any of them.

The animals were received alive. For microscopical examination they were killed by decapitation and the brains prepared either by Nonidez's (1939) silver impregnation technique or by a Nissl method (thionine) following fixation in formol-acetic-alcohol. In each case complete series of sections were cut from paraffin blocks.

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* The expenses of this work were partly met by a grant from the Medical Research Council.

The following series were available for study of the normal brain:

Series no.	Species	Stain	Plane of section	Thickness (μ)
A 7	*A. lineatopus*	Thionine	Transverse	12
A 6	*A. lineatopus*	Thionine	Sagittal	10
A 3*	*A. garmani*	Thionine	Sagittal	15
A 1	*A. lineatopus*	Nonidez	Transverse	7·5
A 4	*A. lineatopus*	Nonidez	Transverse	10
A 5	*A. lineatopus*	Nonidez	Sagittal	10
A 2	*A. garmani*	Nonidez	Transverse	10

* In this specimen the olfactory bulbs and peduncles were sectioned separately in the horizontal plane and stained with thionine.

In an attempt to obtain material showing degeneration effects, olfactory bulbs were removed in three specimens which were prepared as follows:

Series no.	Species	Operation	Survival time (days)	Stain	Plane of section	Thickness (μ)
A 8	*A. grahami*	Unilateral bulbar ablation	2	Nonidez	Transverse	10
A 12	*A. lineatopus*	Bilateral bulbar ablation	2	Nonidez	Transverse	10
A 9	*A. grahami*	Bilateral bulbar ablation	14	Nonidez	Transverse	10

For examination of the peripheral olfactory apparatus, including the vomeronasal organ, transverse serial sections were cut through the whole snout (after decalcification) in specimens A1, A2, A4, A6 and A9. The sections from A2, A4, A6 and A9 were stained with haematoxylin and eosin, and those from A1 by de Castro's method. The latter gave no information about the nasal organs which was not obtainable from the haematoxylin and eosin preparations.

For comparative purposes we had a large number of series of sections through the brains of *Lacerta viridis* and *L. vivipara*, cut in all three primary planes and stained by both Nissl and Nonidez methods. Other lacertilian brains available included transverse Nonidez series of *Anguis fragilis* and *Aristelliger praesignis* and Bielschowsky series of *Gecko verticellatus* cut in all three primary planes. We had also the snouts of several specimens of *Lacerta viridis*, cut serially in the transverse plane and stained with haematoxylin and eosin. They included normal specimens and several killed 10–14 days after unilateral ablation of the main and accessory olfactory bulbs.

PERIPHERAL OLFACTORY APPARATUS, OLFACTORY BULBS AND PEDUNCLES

The nasal sac

This is a fairly simple tubular structure and, as in lizards generally, is divisible into anterior and olfactory chambers (Pratt, 1948; Bellairs, 1949). The anterior chamber is short; it is lined by a thin stratified epithelium which extends into the anterior end of the olfactory chamber. The main part of the olfactory chamber, which contains the olfactory receptors, is usually known as the conchal zone. This term is inappropriate in *Anolis*, for there is no sign of a concha. A thick, sensory epithelium (Pl. 1, fig. 5) is limited to the anterior half of the zone where it covers much of the roof, the entire medial wall and the medial third of the floor. Although relatively much smaller in area, this epithelium is almost identical histologically with the olfactory epithelium in *Lacerta viridis*; it is a little thinner, and Bowman's glands, though present, are small and few in number. Beneath the epithelium many bundles of olfactory nerve fibres can be seen.

The vomero-nasal organ

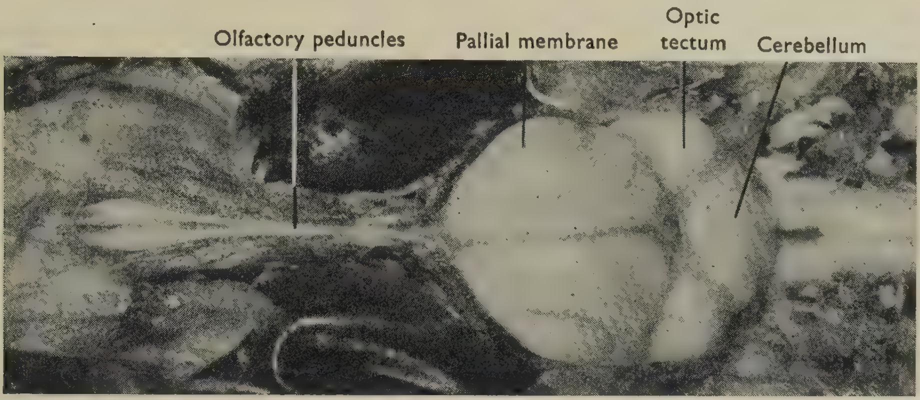
This structure is present but small, as noted by Pratt (1948) in *Anolis alligator*. All the usual components are present but less well-differentiated than in most lizards. Two kinds of lining epithelium can be recognized: a ciliated columnar epithelium covers the so-called mushroom body and a much thicker and entirely different epithelium covers the dorsal and medial walls (Pl. 1, figs. 2, 3). In sections stained with haematoxylin and eosin, three layers can be recognized in the latter: (i) superficially, a faintly striated eosinophilic layer containing only occasional small nuclei, (ii) a narrow layer of large, oval, vesicular nuclei which is succeeded by (iii), a much thicker layer of small and deeply staining nuclei. Bowman's glands are not present. The subepithelial tissue contains several fine nerve bundles, blood vessels and pigmented cells.

In all these features the epithelium under consideration resembles very closely the sensory epithelium of the vomero-nasal organ in *Lacerta viridis*, and indeed of many other vertebrates (Kolmer, 1927); it differs from that of the nasal cavity only in the greater thickness of the third layer, and in the absence of Bowman's glands. The unequivocal identification of neuro-sensory cells and supporting cells is not possible in sections stained only with haematoxylin and eosin, but from what is known in other forms it appears almost certain that the two superficial layers are formed by the outer parts of the cell bodies and by the nuclei of supporting cells, between which the distal processes of the neuro-sensory cells project, and that the deeper or third layer consists of the nuclei and cell bodies of the neuro-sensory cells. As will be shown, our experimental findings are consistent with this interpretation.

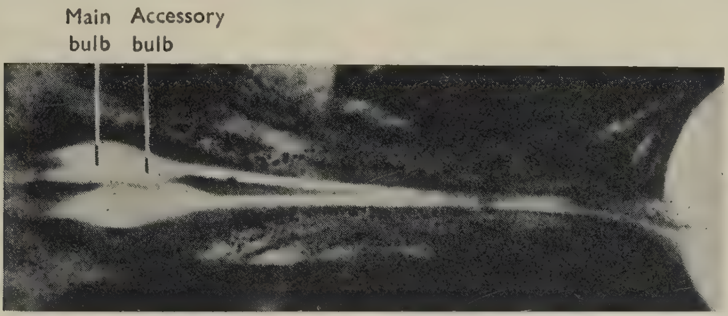
The olfactory bulbs and peduncles

A dorsal view of the exposed brain (Text-fig. 1) shows at once what appear to be olfactory bulbs attached to the cerebral hemispheres by long peduncles in the manner characteristic of the Lacertilia. The bulbs are small both absolutely and relatively, and under low magnification are seen to be divided by a slight depression on the medial side into larger anterior and smaller posterior portions (Text-fig. 2). It would seem natural to identify these with the main and accessory olfactory bulbs of other lizards. The peduncles are very fine, and are attached to the hemispheres just beneath the frontal poles.

The microscopical evidence is entirely consistent with the above interpretation. The anterior part of the bulb complex surrounds a centrally situated olfactory ventricle which, as in reptiles generally, is patent throughout the length of the peduncle. Superficially it is covered by a network of nerve fibres into which the nerve bundles from under the olfactory epithelium of the nasal sac could be traced in two specimens in which the bulbs had been left *in situ.* Beneath this are the 'glomeruli', which are succeeded by neurons of medium size with well-stained Nissl substance. These are the mitral cells. Next is a layer of fine fibres and beneath it a periventricular layer of small densely packed granule cells. The fibres and the layer of granule cells extend caudally into the olfactory peduncle.



Text-fig. 1. Brain of *Anolis garmani, in situ,* exposed from above. (×6.)



Text-fig. 2. Olfactory bulbs and peduncles of *A. garmani* exposed from above, showing the subdivision into main and accessory bulbs. (×11.)

In the caudal part of the complex the olfactory ventricle is displaced laterally, and olfactory bulbar formation is present only in the medial wall. The deeper layers here are continuous with those more rostrally placed, but there is a distinct break in the glomerular layer at a level corresponding to the depression visible externally. The nerve bundles found in relation to the epithelium of the vomero-nasal organ can be traced caudally until they join to form a single nerve which enters this caudal part of the olfactory bulb complex. There is no doubt therefore that it represents a typical accessory olfactory bulb as the gross appearance suggested.

The peduncles call for little comment except to note their extreme fineness. In *Anolis garmani* they are about 0·1 mm. in diameter. They contain small cells of uncertain nature forming the periventricular grey matter, external to which are many fine fibres, running rostro-caudally. These are presumably the axons of mitral and other cells in the bulbs: among them are a few scattered cells most of which are probably neuroglial.

Effects of bulbar ablation

In one specimen (A9) it was possible to examine the effects of bilateral removal of both main and accessory bulbs on the peripheral olfactory apparatus. It was found that 14 days after the operation many of the small cells had disappeared from the deepest layer of the epithelium; this loss was more conspicuous in the thicker epithelium of the vomero-nasal organ (Pl. 1, figs. 3, 4) than in the olfactory epithelium of the nasal sac (Pl. 1, figs. 5, 6), but was quite definite in both. It is interpreted as a retrograde degeneration of neuro-sensory cells in both situations, and is very similar to the secondary degeneration described by Clark & Warwick (1946) in the olfactory epithelium of the rabbit following bulbar ablation; such degeneration does not appear to have been recorded previously in the vomero-nasal organ.

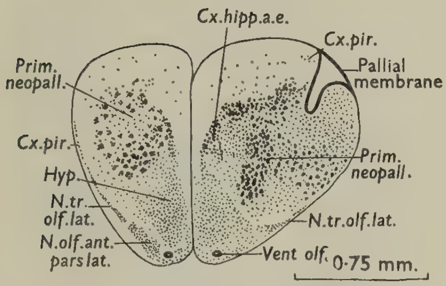
Almost precisely similar changes were seen in the olfactory and vomero-nasal epithelia in the specimens of *Lacerta viridis* examined 10–14 days after unilateral bulbar ablation (Pl. 1, fig. 1). In this species a basal layer of cells, next to the sub-epithelial connective tissue, was unaffected; these may be additional supporting cells, possibly similar to the basal cells mentioned by Clark & Warwick (1946) as persisting in the olfactory epithelium of the rabbit 8 weeks after bulbar ablation.

It is reasonable to conclude that in the species of *Anolis* which have been examined the nasal sac and the vomero-nasal organ both contain typical neuro-sensory epithelium and are connected with main and accessory olfactory bulbs respectively. The epithelium and the olfactory bulbs are atypical only in so far as they are reduced in extent, both absolutely and relatively, in comparison with their development in most Lacertilia. There is no reason to doubt that these organs are functional, nor anything to suggest that their functions differ from those of corresponding organs in other reptiles.

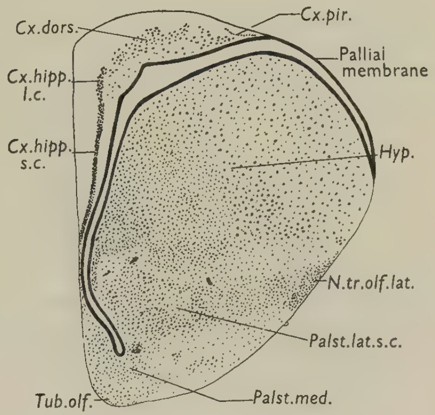
THE CEREBRAL HEMISPHERES

The general form of the hemispheres is shown in Text-fig. 1. They are foreshortened in comparison with the usual lacertilian condition, and more genuinely hemispherical in shape; in this respect they are somewhat reminiscent of the crocodilian forebrain. The point of junction with the olfactory peduncles is overlapped by the frontal pole and is marked by a small conical projection which represents the anterior olfactory nucleus (this is not visible in Text-fig. 1). The crescentic line seen on the dorso-lateral surface in the photograph marks the dorsal and caudal margins of a region of extreme thinning of the pallium, which is reduced in the area enclosed by the line to a transparent membrane. A thinning in this situation, leading to the formation of a 'pallial membrane', appears to be unique among the reptiles so far described. The strio-amygdaloid complex (including the so-called hypopallium) is relatively very large in *Anolis*, and part of its ventricular surface can be seen through the pallial membrane. The general relationships of pallial and basal structures and the pallial membrane are best seen in transverse sections, e.g. Text-figs. 3–8.

Surface fissures of comparative morphological significance are very poorly developed. On the ventro-lateral surface of the hemisphere there is a constant small depression which may represent the caudal end of the endorhinal or amygdaloid fissure. Medially, it is only in the region dorsal to the supraforaminal part of the paraterminal body (or septum) that there is any suggestion of a fissura limitans hippocampi, although in other reptiles such a fissure seems to be a constant finding (de Lange, 1911; Johnston, 1915; Goldby, 1934).



Text-fig. 3.



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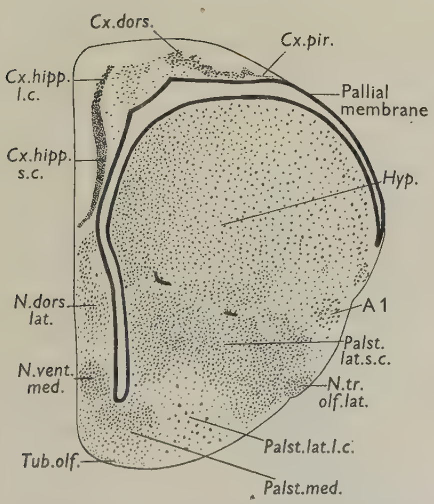
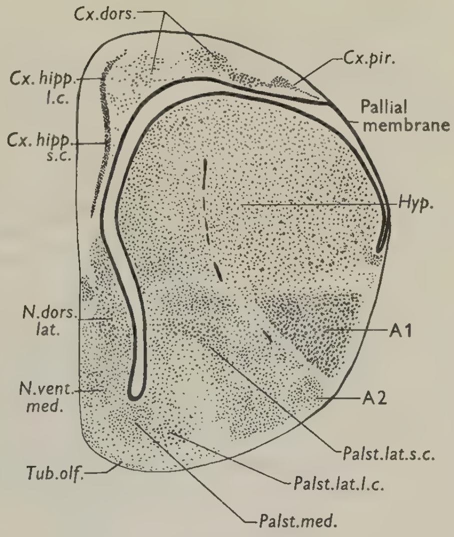


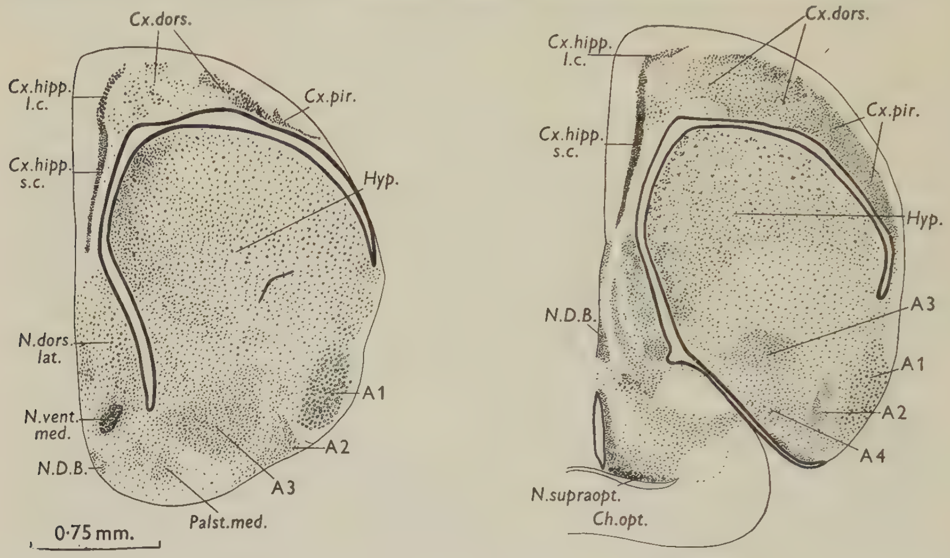
Text-fig. 5.



Text-fig. 6.

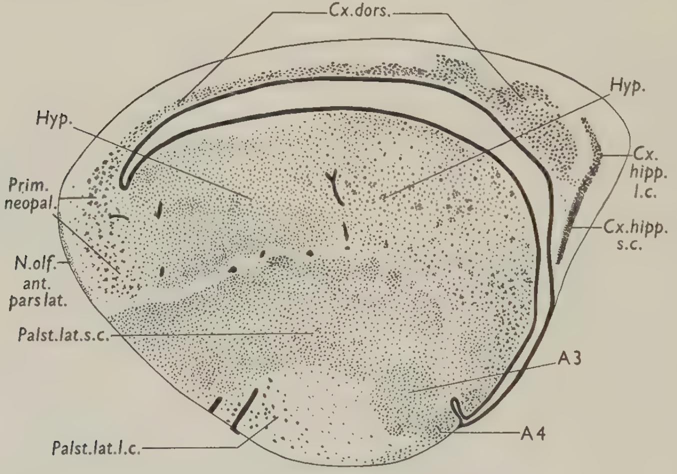
Text-figs. 3–6. Scale drawings of transverse sections through a cerebral hemisphere of *Anolis lineatopus* stained with thionine. For meaning of abbreviations used in all text-figures see p. 307.

Two other points of interest are shown in Text-fig. 1. The very large cerebellum extends rostrally almost to touch the caudal poles of the cerebral hemispheres, and associated with this there is a marked lateral displacement of the optic lobes.



Text-fig. 7. Text-fig. 8.

Text-figs. 7 and 8. Scale drawings of transverse sections through a cerebral hemisphere of *Anolis lineatopus* stained with thionine.



Text-fig. 9.

Text-fig. 9. Scale drawing of a parasagittal section through a cerebral hemisphere of *Anolis garmani* stained with thionine.

Anterior olfactory nucleus

This nucleus is formed by an ill-defined zone of small round cells external to the periventricular grey matter, and appears to correspond with the anterior olfactory nucleus of other lizards such as *Lacerta* (Goldby, 1934) and *Varanus* (Crosby &

Humphrey, 1939*b*). In *Anolis* it shows cellular condensations dorsally and laterally. The lateral condensation is found to be continuous caudally with two cellular strands on the surface of the hemisphere. The first of these is a row of small, oval cells extending dorso-laterally along the rostral and dorsal margin of the pallial membrane (Text-figs. 3, 4, *Cx.Pir.*), forming the anterior extremity of what appears to be an extremely rudimentary piriform cortex. The second is a collection of cells which can be traced caudally in association with the lateral olfactory tract; in some sections these appear to form a definite interstitial nucleus of the tract, in others they appear as an ill-defined collection of cells difficult to distinguish from those of the underlying palaeostriatum lateralis.

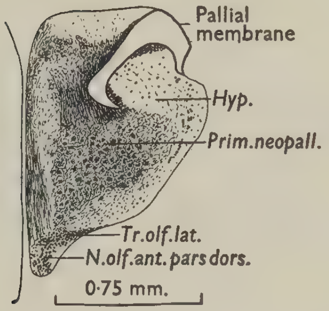
Dorsally the anterior olfactory nucleus has a somewhat tenuous connexion with the anterior extremities of the dorsal and hippocampal cortices, and ventrally it extends as a few small scattered cells overlying the palaeostriatum and forming a very indefinite olfactory tubercle. Medially, the nucleus is represented by only a few scattered cells which are replaced caudally by the anterior end of the paraterminal body. In brief, the anterior olfactory nucleus of *Anolis* is similar to that of other lizards, but is relatively much smaller.

Strio-amygdaloid complex

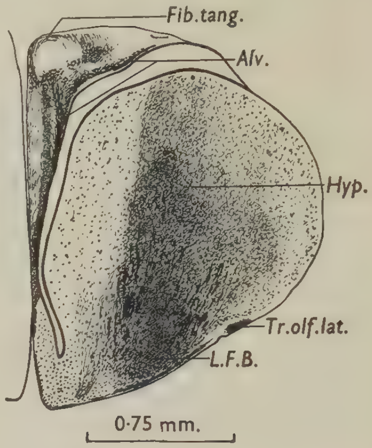
A characteristic feature of the reptilian hemisphere is the large and complex ventro-lateral cell mass which invaginates the lateral ventricle. As the name indicates, this mass develops in the region of the hemisphere wall which is occupied by the corpus striatum and amygdaloid nuclei in mammals. There is considerable uncertainty whether some of the components which can be distinguished in reptiles have any mammalian counterparts, and as a result a cumbersome and inconsistent terminology has grown up in reference to this part of the brain. This terminology was reviewed in detail by Goldby (1934) and by Kappers *et al.* (1936). To clarify the description which follows, it will suffice here to summarize the principal components.

On the basis of more or less well-defined cellular condensations three main components are generally recognized; they are known respectively as the palaeostriatum, the hypopallium and the amygdaloid complex. The palaeostriatum is found rostrally and ventrally and is usually divided into medial and lateral parts, of which the medial extends below the lateral ventricle into the septum as a nucleus accumbens. It lies immediately beneath the surface of the brain in the region of the olfactory tubercle, and its cells are scattered among the fibres of the forebrain bundles. The hypopallium forms the dorsal and rostral part of the region and is usually the largest of its three main components. It commonly appears as a cellular cap immediately beneath the ependyma over the convex dorsal and medial surface. Since it appears to be connected with terminal fibres of the lateral forebrain bundle, it is sometimes grouped with the palaeostriatum as a composite functional unit, the whole being referred to as the 'corpus striatum'.

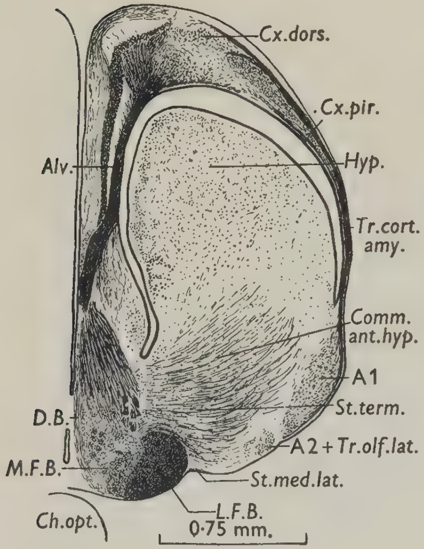
The amygdaloid complex is situated caudally and has characteristic connexions with the lateral olfactory tract and stria terminalis system of fibres. A 'hypopallium posterior' has been identified in many reptiles and interpreted by a number of authors (e.g. Goldby, 1934) as a part of the amygdaloid complex. When well developed, as in many lizards and snakes, it resembles in cellular arrangement and certain topographical relationships the hypopallium described above (to which the term 'anterior' has been applied). Its main connexion, however, is with the lateral olfactory tract and it seems better to refer to it as one of the amygdaloid nuclei than to use a term which implies essential similarity to the hypopallium, as described above, of which there is little evidence. Other terms have been applied to this structure, e.g. 'nucleus sphaericus' and more recently 'posterior amygdaloid nucleus' (Curwen, 1939).



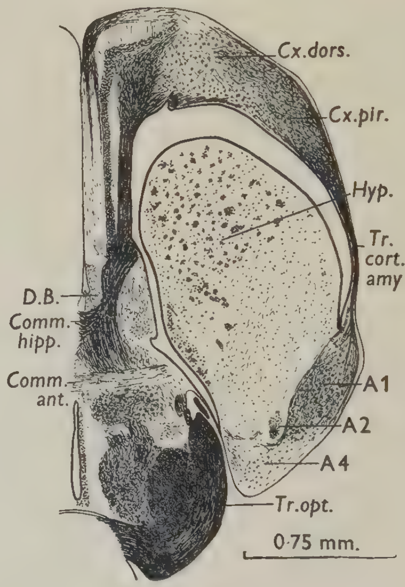
Text-fig. 10.



Text-fig. 11.



Text-fig. 12.



Text-fig. 13.

Text-figs. 10–13. Scale drawings of transverse sections through a cerebral hemisphere of *Anolis lineatopus*. Nonidez's silver impregnation method.

In *Anolis* all three main components of the strio-amygdaloid complex can be readily identified. As a whole it is relatively much larger than in most lizards, due principally to the great size of the hypopallium. Other features are as follows: for the most part only those in which *Anolis* differs from at least the majority of lizards are described.

(*a*) *Palaeostriatum* (Text-figs. 4–6 and 9). In Nissl preparations this is found to be divisible into the usual medial and lateral parts, related to the rostral ends of the medial and lateral forebrain bundles respectively, although in silver impregnated sections the separation of the medial from the lateral bundles in this part of the hemisphere is somewhat arbitrary. The lateral part of the palaeostriatum is further differentiated from the medial by the presence of a dense network of fibres among its cells (Text-fig. 11); it would appear, therefore, to be the main field of distribution for the lateral forebrain bundle, a characteristic which it may share with the overlying rostro-lateral part of the hypopallium.

Most of the cells of the palaeostriatum are small, but there is a conspicuous group of large deeply staining neurons of the motor type ventrally situated in its lateral sub-division (Text-figs. 5, 6 and 9). These cells may give rise to descending fibres in the lateral forebrain bundle, and they suggest comparison with the large cells of the globus pallidus in mammals.

(*b*) *The hypopallium.* This occupies rather more than the dorsal half of the whole strio-amygdaloid complex. Nissl preparations show that the cell pattern varies to some extent within the hypopallium, although there are no clear-cut subdivisions. Thus, in its caudal part there is a tendency for cells to be arranged in clumps near the ventricular surface (Text-figs. 8, 9 and 13), while rostrally the cells are smaller and more closely packed medially than they are laterally (Text-figs. 4–7). The rostro-lateral part of the hypopallium is also characterized by a dense intercellular network of fibres (Text-fig. 11), which in the material available seems to consist partly of terminal fibres from the lateral forebrain bundle and partly of short fibres connecting with the underlying palaeostriatum. In the rostro-medial and caudal parts of the hypopallium very few pericellular fibres are demonstrable, at least by the methods we have used; a few fibres enter the rostro-medial part from the palaeostriatum and perhaps from the lateral forebrain bundle, but caudally the only extrinsic connexion appears to be through small fibre bundles which converge towards the anterior commissure.

(*c*) *The amygdaloid complex.* This complex is fairly large in *Anolis* and shows more definite subdivisions than in many lizards, e.g. *Lacerta*, *Anguis* and the geckos. Four nuclei can be distinguished which will be called A1, A2, A3 and A4 in order to avoid suggestions of homology which might be implied if more descriptive terms were used.

Nucleus A1 is conspicuous and well defined. In the rostral part of the complex, it has the shape of a wedge between the hypopallium above and the caudal part of the palaeostriatum lateralis below (Text-fig. 6); at more caudal levels it assumes a distinctly oval outline in transverse sections (Text-figs. 7, 8, 12 and 13). Its cells are of medium size and multipolar; they stain deeply, and are surrounded by a dense network of fibres. The nucleus is bounded on its ventral and lateral sides by a thin capsule of fibres which converge with some of the intercellular fibres to form a prominent tract which can be followed dorsally along the posterior margin of the pallial membrane, apparently to connect with the caudal parts of the piriform and dorsal cortices. We have named this the cortico-amygdaloid tract (Text-figs. 12, 13). No fibres of the lateral olfactory tract or of the stria terminalis system can be traced to this nucleus, which is included in the amygdala only by reason of its topographical position.

Nucleus A2 (Text-figs. 6–8, 12 and 13) is a collection of small round cells, forming a caudal continuation of the nucleus of the lateral olfactory tract, which passes ventral and medial to nucleus A1. At its caudal extremity it forms a tiny cap of cells around the termination of the lateral olfactory tract, and this part of the nucleus is related to a small ingrowth of ependyma which invaginates the caudal surface of the strio-amygdaloid complex. Nucleus A2 is the only part of the amygdaloid complex which receives olfactory tract fibres directly; although small, and not very clearly defined, its relation to the ependyma and its connexion with the lateral olfactory tract suggest a correspondence with the larger and more clearly differentiated 'hypopallium posterior' of *Lacerta* and most other *Squamata.*

Nucleus A3 is a large mass of small cells immediately caudal to the palaeostriatum in the central and medial parts of the amygdaloid complex (Text-figs. 7–9). Medially it extends dorsal to the lateral forebrain bundle. It shows a dense intercellular neuropil, and the main extrinsic connexions clearly pass to the stria terminalis system and the anterior commissure (Text-fig. 12).

Nucleus A4 lies in the ventro-caudal part of the complex (Text-figs. 8, 9). Its cells are small and resemble those of A3. The connexions of this nucleus appear to be of two kinds; many fibres pass dorso-medially into nucleus A3 and thence into the stria terminalis, while others run dorso-laterally into nucleus A1 and possibly through that nucleus into the cortico-amygdaloid tract (Text-fig. 13).

Paraterminal body

The paraterminal body or septum forms the medial wall of the hemisphere between the hippocampal cortex above and the olfactory tubercle and palaeostriatum medialis below (Text-figs. 5–7). Caudally it forms a bed for the hippocampal and anterior commissures, and extends dorsal to the interventricular foramen as far as the posterior hippocampal commissure.

Rostrally the paraterminal body is very thin (Text-figs. 4, 11). Caudally it is much thicker, forming a characteristic projection into the lateral ventricle (Text-figs. 6, 7); here, as in most reptiles, it shows a subdivision into dorso-lateral and ventro-medial nuclei, the former consisting of larger cells than the latter; neither is very clearly defined. A condensation of small cells, superficially placed immediately anterior to the commissures, represents the nucleus of the diagonal band; ventrally these cells come into close relationship with the large cells of the supraoptic nucleus. Other cellular condensations form bed nuclei for the commissures.

The fibre systems related to this region are very similar to those in *Lacerta viridis.* Dorsally it receives fibres from both the superficial and deep aspects of the hippocampal cortex (tangential and alveus fibres respectively), and ventrally, particularly in its more caudal part, it is very closely related to the medial forebrain bundle.

Whether these fibre systems are afferent or efferent connexions of the paraterminal body is not known, nor how far they may be fibres of passage, passing, for example, from the hippocampus to the medial forebrain bundle or vice versa.

The nucleus of the diagonal band is accompanied by a well-developed bundle of fibres which can be traced from a position superficial to the hippocampal cortex, above the commissures, ventrally to the region of the supraoptic nucleus. These fibres are practically identical with the medial part of the diagonal band system described by Gamble (1952) in *L. viridis*. Rostrally in the paraterminal body there are many fibres, also quite superficial beneath the pia, which run obliquely or longitudinally, and a few of these can be traced ventrally and laterally across the base of the hemisphere to the stria medullaris lateralis. They may correspond in part to the anterior olfacto-habenular tract and the ventral part of the medial olfactory tract of *L. viridis* (Gamble, 1952). In *Anolis* they do not form definite bundles, and cannot be distinguished with confidence from adjacent fibre systems.

Pallium

The most striking feature of the pallium in *Anolis* is the presence of the 'pallial membrane' (Text-figs. 3–7, 10 and 11). This consists of an outer layer of pia mater and an inner layer of flattened ependymal cells, and contains no nerve cells, neuroglia or nerve fibres. It occupies much of that region where the piriform cortical area is found in other reptiles. It is quite separate from another membranous area on the ventro-medial aspect of the hemisphere wall, caudal to the commissures, from part of which the choroid plexus of the lateral ventricle is formed. This ventro-medial membranous area is present in *Anolis* and in most, perhaps all other reptiles; it seems to represent the region of the choroidal fissure in mammals but is less completely invaginated into the ventricle.

Hippocampal and dorsal cortical areas can easily be recognized in *Anolis*. Although differing in some details from these areas in other lizards, they conform to the same general pattern, particularly to that found in *Lacerta viridis*, and show no obvious difference in extent and degree of differentiation. The piriform area is less easily recognizable; it appears to be present in *Anolis* but to be extremely rudimentary, at least in the anterior half of the hemisphere. Some additional features of these cortical areas are as follows.

(*a*) *The hippocampus.* The hippocampal area is situated mainly on the medial aspect of the hemisphere and shows the usual differentiation into large and small-celled parts (Text-figs. 4–9). The small-celled part extends back to form a covering to the whole posterior pole of the hemisphere; otherwise it is entirely confined to the medial surface. The large-celled part is also situated on the medial surface (Text-figs. 4–7) except for a short distance in the caudal part of the hemisphere where it extends on to the dorsal surface (Text-fig. 8). The relatively small extent of the large-celled part on the dorsum of the hemisphere is the only obvious difference in the hippocampus of *Anolis* from that of *Lacerta viridis*.

A well-developed system of tangential fibres lies superficial to the cells of the hippocampal cortex, closely applied to its small-celled part but separated from the large-celled part by a zone of neuropil. This zone which is characteristic of *Squamata* generally contains radially orientated fibres, which seem to connect the tangential fibres with the alveus system of fibres next to the ventricular ependyma. As in other reptiles, the alveus system extends well beyond the hippocampal area and beneath the dorsal cortex.

(*b*) *The dorsal cortex.* This forms an almost continuous sheet with the hippocampus in the anterior part of the hemisphere, but is overlapped by the large-celled hippocampus caudally (Text-figs. 6–9). It consists of pyramidal or polygonal cells like those of the large-celled hippocampus, but slightly smaller and more irregularly scattered. At about the middle of the hemisphere (Text-figs. 5–7) it is divided into medial and lateral parts by a comparatively cell-free zone. Rostrally its cells extend ventrally in front of the lateral ventricle, forming an oval cell mass in close relation to the rostral extremity of the hypopallium (Text-fig. 9). This extension, in which most of the cells are arranged in small groups, resembles closely the so-called 'primordium neopallii' which has been identified in a wide variety of reptiles since the original descriptions of Crosby (1917) and Smith (1919). It is difficult, however, to find definite criteria whether it should be classified as basal or pallial. In *Anolis* the identity of cell-type with that of the dorsal cortex suggests a pallial derivation, but the cells lie in a dense network of fibres which is continued into the lateral part of the hypopallium. It may be reached directly by some fibres from the lateral forebrain bundle.

In addition to the usual relationship to the tangential and alveus fibre systems, the caudal one-third of the dorsal cortex has a strong connexion with the cortico-amygdaloid tract already described (p. 298); the precise extent of this connexion is difficult to determine since the boundary between dorsal and piriform cortices is indefinite in this region. There is no connexion with the anterior commissure, and the lateral forebrain bundle does not reach the dorsal cortex, except possibly in the region of the 'primordium neopallii'.

(*c*) *The piriform cortex.* This appears to be represented in the rostral part of the hemisphere by a narrow strand of small cells along the rostral and dorsal margins of the pallial membrane, overlapping the lateral part of the dorsal cortex (Text-figs. 4, 5). It can be followed rostrally into continuity with the lateral part of the anterior olfactory nucleus, and some fibres from the lateral olfactory tract can be traced to its superficial surface. Caudally it broadens and becomes considerably thicker (Text-fig. 8), and is no longer clearly separated from the dorsal cortex. At the caudal pole of the hemisphere it becomes continuous with the small-celled hippocampus. The caudal part of the piriform cortex, like the dorsal cortex, is very closely related to the cortico-amygdaloid tract (Text-figs. 12, 13).

Olfactory tracts

In general the secondary and tertiary olfactory connexions are similar to those which have been seen in *Lacerta viridis* (Goldby, 1934), but are more difficult to demonstrate since the fibres are very fine and comparatively few in number.

From the peduncle, fibres can be traced to the anterior olfactory nucleus in which the majority collect laterally. Here they are probably reinforced by additional fibres arising in the anterior olfactory nucleus itself. These lateral fibres can be followed into a small but definite lateral olfactory tract, some fibres from which enter the neuropil overlying the strand of cells identified as piriform cortex. The main part of the tract passes back to the amygdaloid region (Text-figs. 11, 12); it runs ventral to nucleus A1 and, leaving the surface of the brain, enters nucleus A2. Its termination in that nucleus has already been described (p. 298).

Intermediate and medial olfactory tracts can also be identified, although the fibres concerned are so diffusely spread that the term 'tract' is hardly justified. The intermediate tract is represented by fibres which pass through the ventral part of the anterior olfactory nucleus to be distributed over the surface of the olfactory tubercle. Fibres resembling both dorsal and ventral parts of the medial olfactory tract described in *L. viridis* by Gamble (1952) are present; those corresponding to the pars ventralis can be traced caudally and then laterally across the base of the hemisphere to join a very small stria medullaris lateralis. Yet other fibres in this situation may represent the so-called anterior olfacto-habenular tract, which in *L. viridis* was shown by Gamble (1952) to be a well-developed crossed secondary olfactory pathway; in *Anolis* they do not form a separate and compact bundle.

Some additional information was obtained from examining the effects of bulbar ablation. Fourteen days after removal of both bulbs fibres had completely disappeared from the olfactory peduncles, and there was some loss of fibres from among the cells of the anterior olfactory nucleus. Behind this nucleus there was marked loss of fibres from the surface of the olfactory tubercle, and possibly a slight loss from the lateral olfactory tract. It was obvious that the majority of fibres in this tract arise from cells posterior to the bulb, probably in the anterior olfactory nucleus.

Chief interest lies in the possibility of establishing the presence in *Anolis* of a crossed secondary olfactory pathway such as that which exists in *Lacerta viridis*. Unfortunately, the evidence obtained is equivocal. There seemed to be a definite loss of fibres passing across the base of the hemisphere between the stria medullaris lateralis and the surface of the paraterminal body but no loss was demonstrable in the stria medullaris itself nor in the unusually small habenular commissure.

In the two specimens allowed to survive 2 days after bulbar ablation, fragmentation of fibres could be seen superficial to the anterior olfactory nucleus and over the anterior part of the piriform cortex, thus providing support for the identification of that cortex. No further evidence relevant to this investigation could be obtained from these specimens.

DISCUSSION

In the three species of *Anolis* at our disposal the peripheral olfactory apparatus (including the vomero-nasal organ) does not differ fundamentally from that found in other lizards, but the term 'microsmatic' is nevertheless justified. The nasal sac is less complex than usual and the area of its lining epithelium is smaller, while a corresponding reduction in size is found in the vomero-nasal organ. At the same time, the existence of functional sensory epithelium in both chambers is established and this is clearly inconsistent with Pratt's (1948) description of the nasal sac in *A. alligator* as 'almost non-sensory' and of the vomero-nasal organ as 'completely non-sensory'. It is just possible that a species variation is responsible for the discrepancy. It seems more likely, however, that Pratt was influenced in his interpretation by an earlier assertion that no accessory olfactory bulb and only a very rudimentary main bulb could be demonstrated in *Anolis* (Crosby & Humphrey, 1939*a*, *b*); certainly he related these earlier findings to his own.

In view of the relatively reduced condition of the peripheral olfactory organs it is not surprising that the olfactory bulb complex in *Anolis* should be small. Our findings, however, differ fundamentally from those of Kappers *et al.* (1936) and Crosby & Humphrey (1939*a*, *b*). They described the olfactory bulb in *A. carolinensis* as a minute body beneath the frontal pole of the cerebral hemisphere which 'would be missed entirely if it were not so closely associated with the main mass of the forebrain' (Crosby & Humphrey, 1939*a*). Similar findings were reported in *Phrynosoma cornutum* and *Holbrookia maculata*, and no accessory olfactory bulb was recognized in any of these lizards. By way of contrast, the three species of *Anolis* which we have studied possess both main and accessory bulbs. They lie well in front of the hemispheres, and apart from being relatively small do not differ in any essential features from the olfactory bulbs of other lizards.

It seemed that these conflicting accounts might be explained by the fact that different species of *Anolis* were involved; but this possibility was rejected after reference to a paper by Willard (1915) on the cranial nerves in *A. carolinensis.* Although not referred to in the text, the olfactory bulbs are clearly shown in Willard's pl. 2, fig. 4, and pl. 3, fig. 6, and are similar to those described in the present paper. We think, therefore, that the descriptions given by Kappers *et al.* and by Crosby & Humphrey were based upon a misinterpretation. The structure identified by them as an olfactory bulb did not have the histological characteristics of typical bulbar formation, and corresponds topographically with the root of the olfactory peduncle. Their material, presumably, was removed from the head in such a way as to leave behind the olfactory bulb complex and the greater part of the olfactory peduncles. No specimens of *Phrynosoma* or *Holbrookia* were available for the present investigation, but re-examination of the olfactory system in these iguanids is desirable in view of the present findings in *Anolis.*

The central connexions of the olfactory and accessory olfactory bulbs call for little further comment. The general pattern is similar to that in other lizards, although the number of fibres involved in any of the so-called olfactory tracts is clearly much smaller than is usually present. The 'olfactory component' of the anterior commissure could not be recognized at all.

Collections of cells related to the secondary and tertiary olfactory pathways are also relatively inconspicuous in *Anolis.* This is particularly noteworthy in the case of cell groups related to the terminal fibres of the lateral olfactory tract; the anterior part of the piriform cortex is reduced to an almost insignificant remnant, and the main nucleus of termination of the lateral olfactory tract in the amygdaloid region, the 'hypopallium posterior' of many authors, is not recognizable as such. It is represented in *Anolis* by the small nucleus A2. In this context it is worth pointing out that the hippocampal cortical areas, the septal nuclei and the palaeostriatum medialis (sometimes referred to as an 'olfacto-striatum') are all well developed, and show no general reduction or deficiency in their differentiation as compared with other reptiles. Evidently they have not been affected by the reduction of olfactory connexions.

The cause of the reduction of structures concerned with olfaction is of course not indicated by studies of the kind reported here. Bellairs & Boyd (1951) have suggested that a reduction of the peripheral olfactory apparatus may have been related to the adoption of an aboreal mode of life by ancestral iguanid lizards, although other factors may also be of importance since Crosby & Humphrey (1939*a*, *b*) and Stebbins (1948) have observed a similar reduction in the desert-living *Phrynosoma* and in other non-arboreal species. Relatively poor development of the olfactory system seems to be characteristic of many iguanids and perhaps of the whole family; it is not a feature acquired only by the recent arboreal forms.

The fact that reduction of the peripheral receptors and the parts of the forebrain which receive connexions from them go together is, of course, not unexpected. Ontogenetically, it has been shown by Burr (1916) that removal of an olfactory placode in an amphibian embryo results in subnormal development of the olfactory regions in the corresponding hemisphere. It appears that Lopaschov (1937) has also shown that the forebrain, acting as a second-grade organizer, induces the formation of the olfactory placodes. The relationship between peripheral receptor and the forebrain is therefore reciprocal; changes in any part of the complex will affect the whole, and this will apply as much to phylogenetic as to ontogenetic development, since a phylogenetic series must consist of a succession of ontogenies. It is often assumed, however, that olfactory connexions are very widespread in the forebrain, and have been of fundamental importance in the determination of forebrain morphology as a whole. While this may be true in early phylogenetic stages, it is clear that at the reptilian level a reduction of the olfactory apparatus can occur without obviously related changes in any part of the brain except those limited regions to which secondary olfactory fibres have been traced in experimental material (Goldby, 1937; Gamble, 1952). The present observations in *Anolis* therefore support the idea, implicit in a former paper (Gamble, 1952), that olfactory connexions are much less important than is commonly supposed in relation to reptilian forebrain morphology.

The telencephalon as a whole has been treated rather briefly in the descriptive section of this paper. From the results obtained it is clear that it shows in general a typical reptilian pattern. The large size of the ventro-lateral basal centres and the reduction of part of the pallium to a membrane without nervous constituents, suggest developments parallel to those which have occurred in the bird's brain. Similar avian trends were noted in the large size of the cerebellum and the lateral displacement of the optic lobes. These features, however, do no more than emphasize the fact that the reptilian brain in general is related to the avian brain far more closely than to the mammalian.

Differences from other reptiles were, of course, observed. In the pallial and paraterminal regions, with the exception of the reduction of the anterior piriform cortex, these are of a minor character. In the ventro-lateral basal structures they are more marked. The amygdaloid complex shows more intrinsic differentiation than in many other lizards, including *Lacerta* and the geckos. Although lacking a large and highly differentiated 'hypopallium posterior', it resembles that of *Tupinambis*. In this lizard amygdaloid nuclei were classified by Curwen (1939) in two groups: (i) lateral, closely related to the piriform and dorsal cortices by fibres forming a 'primordial external capsule' and (ii) medial, with connexions from the lateral olfactory tract, anterior commissure and stria terminalis. A similar grouping is applicable in *Anolis.* Nucleus A1 and the lateral part of A4 seems to correspond with Curwen's lateral group, and the cortico-amygdaloid tract in *Anolis* is very like her 'primordial external capsule'; similarly, the medial group is represented by nuclei A2, A3, the medial part of A4 and the nucleus of the lateral olfactory tract. The medial group of amygdaloid nuclei is apparently present in all lizards, but the lateral group with cortical connexions has not been generally recognized. It cannot be identified with certainty in any of the other lacertilian material at our disposal though it may be represented in the lateral part of the diffuse 'central amygdaloid mass' described in *Lacerta viridis* by Goldby (1934).

The remainder of the strio-amygdaloid complex has been divided as usual into a ventral palaeostriatum and a dorsal hypopallium. While this subdivision is supported by the appearance of Nissl preparations it is relevant to point out that a division of this whole region into medial and lateral parts would be more in keeping with the appearance of sections in which fibres have been stained. No conclusion can be drawn from these observations, except that, with the evidence at present available, any subdivision in this region must be regarded as somewhat arbitrary. Much the same applies to the identification of a 'primordium neopallii' in *Anolis*, or for that matter in any other reptile. This structure has none of the characteristics by which the neopallium of mammals can be recognized; the claim that it is a primordium of the neopallium rests only on the fact that it occupies a position where the neopallium might have begun to differentiate, and that the fibres of the lateral forebrain bundle which appear to reach it may be ascending from the thalamus and so represent a primitive internal capsule system.

It is becoming increasingly evident that the terminology commonly used in the description of the reptilian brain must be accepted with reserve. Such terms as 'striatum', 'hippocampus', 'amygdaloid' and so on, are taken from human anatomy, and when applied to brains other than the human they lose their character as simple descriptive labels for particular structures, and come to imply an essential similarity. It is important to be aware of just how much similarity the use of these terms in comparative investigations does imply. In some regions, such as the paraterminal or septal region, the topographical position, intrinsic differentiation and relationship to fibre tracts is so similar in all tetrapod vertebrates, that the correspondence is obvious and the use of a common term justified. In others, such as the amygdaloid region, the position is very different. Here the term means little more than that the structure in question always occupies a similar topographical position in the telencephalon. Intrinsic differentiation may be very different, and though a relationship to the lateral olfactory tract and stria terminalis is always present, too little is known of the components and connexions of these fibre systems for this to have much significance.

To give every definable structure, for which there is no unequivocal representative in the mammalian brain, a new and non-committal name may be desirable, but would lead to a very cumbersome terminology. This is unnecessary provided it is realized that the use of terms like 'amygdaloid' imply little more than a broad classification based on topographical location in the wall of the hemisphere vesicle. If one is to go further than this it is necessary to use methods which will give more precise and detailed information about neurons and their connexions than can be given by simple cell and fibre stains in normal material. From their inherent limitations, these methods are incapable of giving more than a preliminary classification in somewhat vaguely defined topographical regions. Their use as a basis for the phylogenetic implications of such terms as 'primordium neopallii' and 'palaeostriatum' is of very doubtful validity. Nevertheless, provided it is realized that the terms are highly speculative, their use can be justified if it stimulates further work on the morphogenesis of the nervous system by methods capable of giving evidence more directly relevant to these problems. In short, the terms used here and in other similar descriptions should in no case be regarded as final and definitive descriptions of the structures to which they are applied, but rather as indicating hypotheses which will be substantiated, modified or perhaps discarded in the light of future work in this field.

SUMMARY

The vomero-nasal organ, olfactory epithelium and telencephalon of *Anolis lineatopus*, *A. garmani* and *A. grahami* have been examined histologically in normal specimens, and at varying intervals after the removal of the olfactory and accessory olfactory bulbs.

The normal material shows a typical neuro-sensory epithelium in the vomero-nasal organ and in the olfactory chamber of the nasal sac which, in both situations, is less extensive than in most reptiles. In the telencephalon small olfactory and accessory olfactory bulbs are present attached to the hemispheres by long slender peduncles in the characteristic lacertilian manner.

In the hemispheres themselves the strio-amygdaloid complex is relatively very large and shows the usual reptilian differentiation into hypopallium, palaeostriatum, and a complex of nuclei in the amygdaloid region; among the latter no 'hypopallium posterior' can be recognized, but it appears to be represented by a small collection of cells receiving fibres from the lateral olfactory tract. In the pallial region the area occupied by the piriform cortex in most reptiles is largely occupied by a thin membrane containing neither nerve cells nor fibres, and the anterior part of the piriform cortex is reduced to a vestige. Dorsal and hippocampal cortical areas, and the septal nuclei, differ only in details from the comparable structures in other lizards. The anterior olfactory nucleus is present but small, and reduced representatives of the usual olfactory tracts are found.

Removal of the olfactory and accessory olfactory bulbs results in degeneration of neuro-sensory cells in the epithelium of both the vomero-nasal organ and the nasal sac, and some fragmentation and loss of fibres in the olfactory tracts.

We wish to express our thanks to Mr Garth Underwood of the University College of the West Indies who collected and sent the live specimens of *Anolis* on which this work is based; we are also indebted to the Photographic Department, St Mary's Hospital Medical School, for the photograph reproduced as Text-fig. 1.

ADDENDUM

Since this paper was sent to press we have found a further reference to the brain of *Anolis lineatopus* in a paper by E. Horne Craigie entitled 'Notes on Cytoarchitectural features of the lateral cortex and related parts of the cerebral hemisphere in a series of reptiles and birds' (*Trans. Roy. Soc. Can.* series 3, **30**, v, pp. 87–113). Craigie gives a description of the pallial membrane, and also notes the vestigial character of the piriform cortical area. He relates this condition to the reduction of the dorsilateral region of the cortex in the bird's brain, but makes no reference to the microsmatic character of *Anolis*. He also gives a brief account of the cell masses which can be differentiated in the ventro-lateral region of the hemisphere, but does not describe them in detail.

REFERENCES

Bellairs, A. d'A. (1949). Observations on the snout of *Varanus*, and a comparison with that of other lizards and snakes. *J. Anat., Lond.*, **83**, 116–146.

Bellairs, A. d'A. & Boyd, J. D. (1950). The lachrymal apparatus in lizards and snakes. II. The anterior part of the lachrymal duct and its relationship with the palate and with the nasal vomero nasal organs. *Proc. zool. Soc. Lond.* **120**, 269–310.

Burr, H. S. (1916). The effects of the removal of the nasal pits in *Amblystoma* embryos. *J. exp. Zool.* **20**, 27–57.

Clark, W. E. le Gros & Warwick, R. T. T. (1946). The pattern of olfactory innervation. *J. Neurol. Psychiat.* **9**, 101–111.

Crosby, Elizabeth Caroline (1917). The forebrain of *Alligator mississippiensis*. *J. comp. Neurol.* **27**, 325–402.

Crosby, Elizabeth Caroline & Humphrey, Tryphena (1939 *a*). A comparison of the olfactory and the accessory olfactory bulbs in certain representative vertebrates. *Pap. Mich. Acad. Sci.* (Part II), **24**, 95–104.

Crosby, Elizabeth Caroline & Humphrey, Tryphena (1939 *b*). Studies of the vertebrate telencephalon. I. The nuclear configuration of the olfactory and accessory olfactory formations and of the nucleus olfactorius anterior in certain reptiles, birds and mammals. *J. comp. Neurol.* **71**, 121–213.

Curwen, Alice Osborne (1939). The telencephalon of *Tupinambis nigropunctatus*. III. Amygdala. *J. comp. Neurol.* **71**, 613–636.

Gamble, H. J. (1952). An experimental study of the secondary olfactory connexions in *Lacerta viridis*. *J. Anat., Lond.*, **86**, 180–196.

Goldby, F. (1934). The cerebral hemispheres of *Lacerta viridis*. *J. Anat., Lond.*, **68**, 157–215.

Goldby, F. (1937). An experimental investigation of the cerebral hemispheres of *Lacerta viridis*. *J. Anat., Lond.*, **71**, 332–355.

Huber, G. C. & Crosby, Elizabeth Caroline (1933). The reptilian optic tectum. *J. comp. Neurol.* **57**, 57–163.

Johnston, J. B. (1915). The cell masses in the forebrain of the turtle, *Cistudo carolina*. *J. comp. Neurol.* **25**, 393–468.

Kappers, C. U. Arëns, Huber, G. C. & Crosby, Elizabeth Caroline (1936). *The Comparative Anatomy of the Nervous System of Vertebrates, including Man*, **2**. New York: The Macmillan Co.

Kolmer, W. (1927). Geruchsorgan. In *Handbuch der Mikroskopischen Anatomie des Menschen*, **3**. (Ed. W. von Möllendorff.) Berlin: Julius Springer.

Lange, S. J. de (1911). Das Vorderhirn der Reptilien. *Folia neuro-biol., Lpz.*, **5**, 548–597.

Lopaschov, G. U. (1937). Quoted by Needham, J. (1942). *Biochemistry and Morphogenesis.* Cambridge University Press.

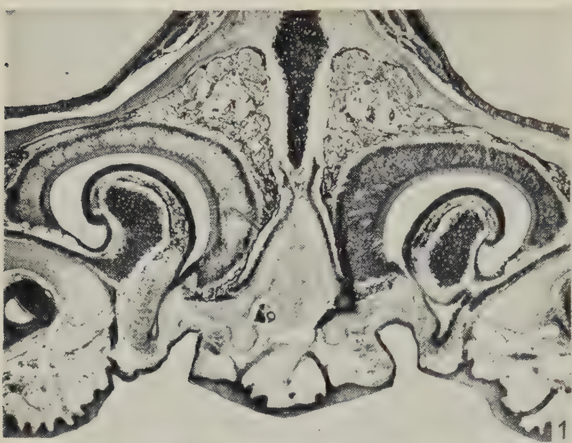
Nonidez, J. F. (1939). Studies on the innervation of the heart. I. Distribution of the cardiac nerves with special reference to the identification of the sympathetic and parasympathetic postganglionics. *Amer. J. Anat.* **65**, 361–413.

Pratt, C. W. M. (1948). The morphology of the ethmoidal region of *Sphenodon* and lizards. *Proc. zool. Soc. Lond.* **118**, 171–201.

Smith, G. Elliot (1919). A preliminary note on the morphology of the corpus striatum and the origin of the neopallium. *J. Anat., Lond.*, **53**, 271–291.

Stebbins, R. C. (1948). Nasal structure in lizards with reference to olfaction and conditioning of the inspired air. *Amer. J. Anat.* **83**, 183–221.

Willard, W. A. (1915). The cranial nerves of *Anolis carolinensis*. *Bull. Mus. comp. Zool. Harv.* **59**, 17–116.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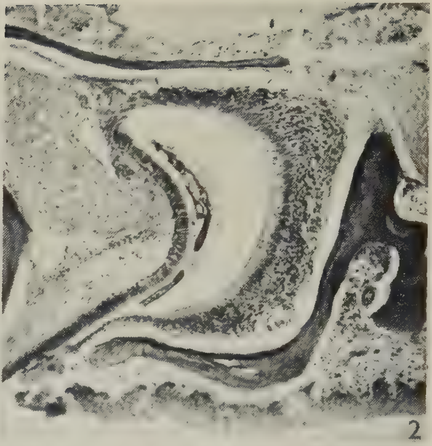
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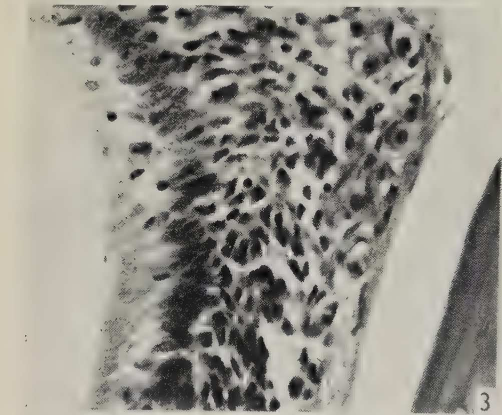
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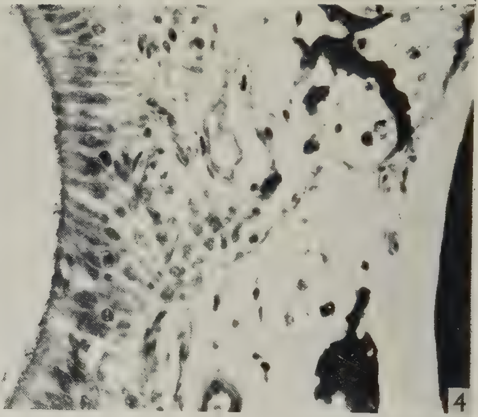
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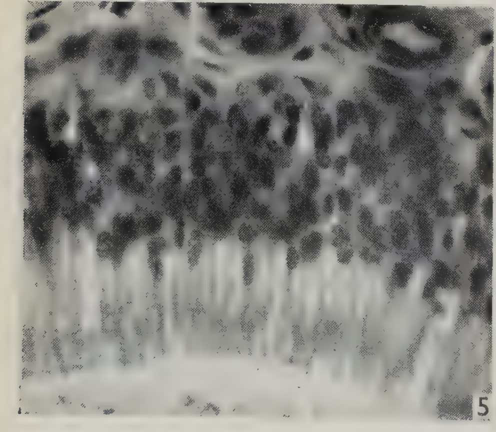
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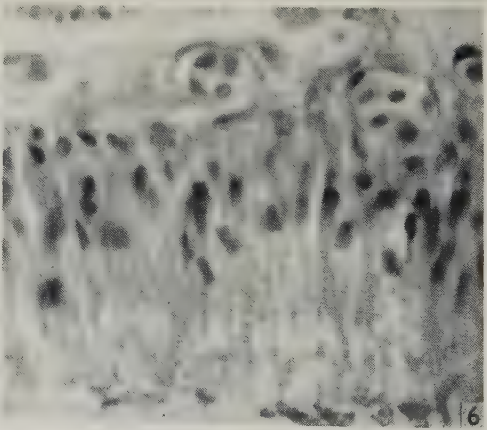
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ARMSTRONG, GAMBLE AND GOLDBY—OLFACTORY APPARATUS AND THE TELENCEPHALON OF *ANOLIS*

EXPLANATION OF PLATE

Fig. 1. Transverse section through the palatal region of *Lacerta viridis* 10 days after unilateral ablation of main and accessory olfactory bulbs. The section shows loss of nuclei from the third layer of the sensory epithelium of the vomero-nasal organ (left side) with persisting basal and supporting cells. Haematoxylin and eosin. (×32.)

Fig. 2. Normal vomero-nasal organ of *Anolis lineatopus*. Transverse section. Haematoxylin and eosin. (×90.)

Fig. 3. Normal sensory epithelium of the vomero-nasal organ in *A. lineatopus*. Haematoxylin and eosin. (×410.)

Fig. 4. Sensory epithelium of the vomero-nasal organ in *A. grahami* 14 days after bilateral ablation of main and accessory olfactory bulbs. The epithelium is much thinner than normal (compare fig. 3), due mainly to a loss of cells from the third layer. Haematoxylin and eosin. (×410.)

Fig. 5. Normal sensory epithelium of the nasal sac in *A. lineatopus*. Haematoxylin and eosin. (×630.)

Fig. 6. Sensory epithelium of the nasal sac in *A. grahami* 14 days after bilateral ablation of main and accessory olfactory bulbs, showing similar changes to those seen in the vomero-nasal epithelium in fig. 4 above. Haematoxylin and eosin. (×630.)

LIST OF ABBREVIATIONS USED IN TEXT-FIGURES

A1–A4	Amygdaloid nuclei
Alv.	Alveus
Ch.opt.	Optic chiasma
Comm.ant.	Anterior commissure
Comm.ant.hyp.	Hypopallial component of anterior commissure
Comm.hipp.	Hippocampal commissure
Cx.dors.	Dorsal cortex
Cx.hipp.a.e.	Hippocampal cortex, anterior extremity
Cx.hipp.l.c.	Hippocampal cortex, large-celled part
Cx.hipp.s.c.	Hippocampal cortex, small-celled part
Cx.pir.	Piriform cortex
D.B.	Diagonal band
Fib.tang.	Fibrae tangentiales
Hyp.	Hypopallium
L.F.B.	Lateral forebrain bundle
M.F.B.	Medial forebrain bundle
N.D.B.	Nucleus of diagonal band
N.dors.lat.	Dorso-lateral nucleus of paraterminal body
N.olf.ant. pars lat.	Anterior olfactory nucleus, pars lateralis
N.olf.ant. pars dors.	Anterior olfactory nucleus, pars dorsalis
N.supraopt.	Supraoptic nucleus
N.tr.olf.lat.	Nucleus of the lateral olfactory tract
N.vent.med.	Ventro-medial nucleus of paraterminal body
Palst.lat.l.c.	Palaeostriatum lateralis, large-celled part
Palst.lat.s.c.	Palaeostriatum lateralis, small-celled part
Palst.med.	Palaeostriatum medialis
Prim.neopall.	Primordium neopallii
St.med.lat.	Stria medullaris lateralis
St.term.	Stria terminalis
Tr.cort.amy.	Cortico-amygdaloid tract
Tr.olf.lat.	Lateral olfactory tract
Tr.opt.	Optic tract
Tub.olf.	Olfactory tubercle
Vent.olf.	Olfactory ventricle

THE DEEP FLEXORS OF THE FOOT

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Attention was drawn some time ago (Barlow, 1949) to an anomalous condition of the flexor digitorum longus in which the whole of the tendon to the little toe, and most of the tendinous fibres to the fourth toe, were arising from flexor accessorius. This interesting finding was adduced as evidence in support of the phylogenetic history of the deep flexor muscles of the foot as interpreted by Wood Jones (1944), and further evidence has now accumulated which can only strengthen the claims of this developmental theory. This evidence consists of three further specimens in which there are varying degrees of direct contribution to the deep flexor tendons by the flexor accessorius. In the first two instances the specimens were from different individuals, the condition being unilateral and the other foot normal; but the complete divorcement of flexor digitorum longus from the toes described in the third specimen was bilateral.

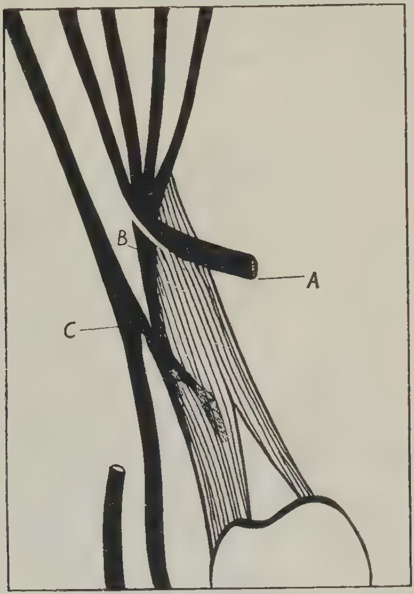

Fig. 1.

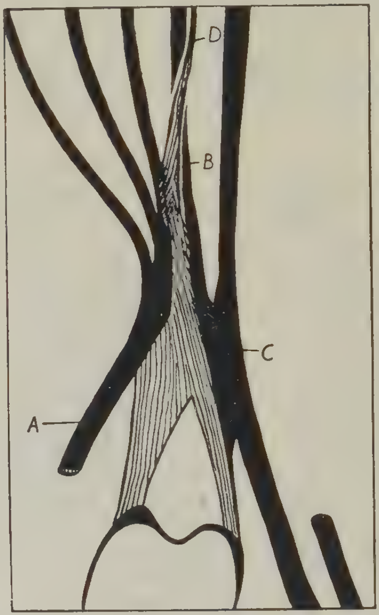

Fig. 2.

Fig. 1. Left foot. *A*, flexor digitorum longus turned laterally; *B*, tendon from accessorius to the lateral three toes; *C*, flexor hallucis longus with small tendinous attachment from accessorius.

Fig. 2. Right foot. *A*, flexor digitorum longus turned laterally; *B*, tendon to second toe arising from accessorius; *C*, flexor hallucis longus with broad tendinous attachment to accessorius; *D*, first lumbrical (others normal).

In the first foot a tendon springs from the medial aspect of flexor accessorius and contributes approximately half the fibres to the tendons of the 3rd, 4th and 5th toes, and sends a small offshoot to flexor hallucis longus (Fig. 1).

In the second foot the tendon of flexor digitorum longus to the second toe has been practically replaced by a new tendon arising from flexor accessorius. Here, too, a broad thin tendinous link with flexor hallucis longus exists (Fig. 2).

The third specimen is most interesting of all, for in it all the deep flexor tendons are arising from the flexor accessorius (Fig. 3). This foot merits a little fuller description. All the deep leg muscles are present in the posterior aspect of the calf, and their tendons run normal courses to the medial side of the ankle. Here tibialis posterior passes to its normal insertion (Fig. 3). The tendons of flexor digitorum longus and flexor hallucis longus pass round the medial side of the ankle and lose themselves in a mass of fibrous tissue on the medial side of the foot situated in the hollow of the calcaneum, below the sustentaculum tali (Fig. 3). From the lateral side of this mass flexor accessorius arises, giving origin to the four tendons normally derived from flexor digitorum longus. The usual small lateral head of flexor accessorius is absent. An entirely separate flexor hallucis longus arises from the medial tubercle of the calcaneum as indicated in Fig. 3, its tendon passing forward to the usual insertion in the base of the distal phalanx of the hallux.

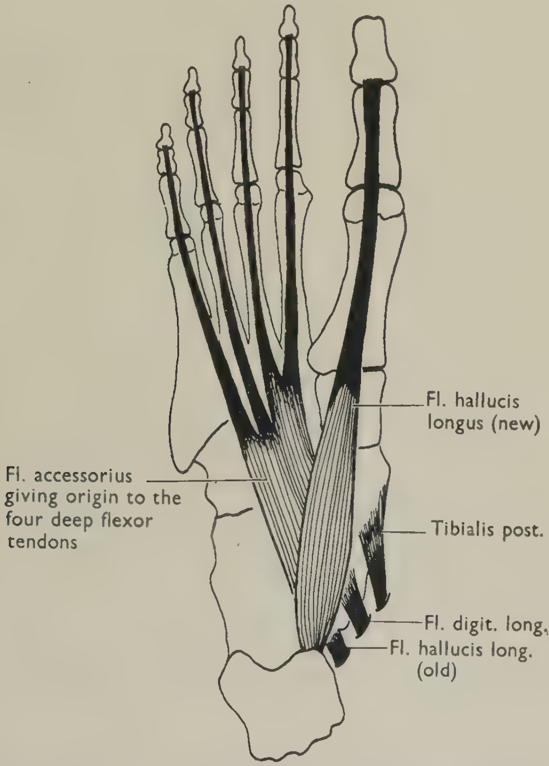
Fl. hallucis longus (new)
Fl. accessorius giving origin to the four deep flexor tendons
Tibialis post.
Fl. digit. long.
Fl. hallucis long. (old)

Fig. 3. Right foot. Complete divorcement of leg flexors from foot muscles showing flexor accessorius and the new plantar portion of flexor hallucis longus.

Wood Jones (1944) suggested that the phylogenetic trend in the foot was toward separation of the musculature of the foot from that of the calf in the interests of efficiency in walking, instancing the superficial flexor, and flexor accessorius as examples. The fact that flexor accessorius can take over the role of the deep flexor completely is, therefore, of considerable importance, but whether this is an evolutionary trend or not only succeeding generations of anatomists will be able to determine.

SUMMARY

Further anomalies of the deep flexors of the foot are described, and their evolutionary significance discussed with reason for supposing that the changes are progressive.

REFERENCES

Barlow, T. E. (1949). An unusual anomaly of m. flexor digitorum longus. *J. Anat., Lond.*, **83**, 224–226.

Jones, F. Wood (1944). *Structure and function as seen in the Foot.* London: Baillière, Tindall and Cox.

STUDIES OF THE VASCULAR ARRANGEMENTS OF THE NOSE

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For the past 100 years the study of nasal angioarchitecture has been largely devoted to an investigation of the 'cavernous' or 'erectile' tissue of the nose in man, following the short description by Kohlrausch (1853). Zuckerkandl (1885) laid the foundation stone for all subsequent studies, but up to the present time investigations have been carried out almost entirely in man. Swindle (1935, 1937) is the only worker who has made a comprehensive study of the vascular arrangements of the nose in animals, but his chief contribution was to demonstrate that in some species the veins lie superficial to the arteries, while in other species the arteries lie superficial to the veins. We have been unable to find in the literature any detailed account of the pattern and distribution of the nasal blood vessels in the animals which are commonly used in experimental investigations.

Since Sucquet (1862) postulated the existence of direct communications between arteries and veins in the nasal mucosa as a result of his injection experiments, various workers have examined the nose to see whether or not these structures are present. The work of Guaragna (1940) and Bachi (1947) suggests that arterio-venous anastomoses are to be found in the inferior turbinate of man, a site in which Fabbi & Rossatti (1951) conclusively demonstrated their existence. Harper (1949) stated that he had observed arterio-venous anastomoses in the mucosa of the human inferior turbinate, and Märk (1941) showed that they were present in the middle turbinate of man. There is little information as to whether arterio-venous anastomoses exist in the nasal mucosa of species other than man, though Harper (1949) reported finding an unusual type of anastomosis in the turbinate mucosa of certain ungulates. Accordingly, one of the objects of this investigation was to try to determine whether or not these structures are present in the nasal mucosa of various animals.

METHODS

The following animals have been used in this investigation: 23 dogs, 19 cats, 18 rabbits, 4 rats, 6 goats, 3 sheep and 2 pigs. To provide data for another research the study was mainly carried out on the dog, the other species being used chiefly for a comparative study of certain features. The material was obtained post-mortem, the animals having usually been killed by bleeding. Most of the specimens were injected, the methods used being similar to those described by Trueta, Barclay, Daniel, Franklin & Prichard (1947, pp. 43–47). Two types of injection mass were employed, namely, neoprene latex to make casts of the vessels, and other masses to demonstrate the vessels as they lie within the tissue. The injection mass was introduced into either the common or external carotid artery or, in a few instances, directly into the maxillary artery. Bilateral injections were sometimes made.

Neoprene latex was injected from a pressure bottle at pressures ranging from 200 to 500 mm. Hg, and the tissue was removed by maceration in concentrated HCl. If fat remained after maceration in acid the cast was placed in a 10 % solution of KOH. The casts were dissected under water and examined with a stereoscopic binocular microscope.

Some specimens were injected with a suspension of barium sulphate, and various regions of these preparations were X-rayed. Other specimens were injected with Berlin blue (2 % in distilled water) or Higgins's indian ink (one part to two of distilled water). Injected specimens were examined in several types of preparation. Some blocks of tissue were cleared by the Spalteholz method. Other blocks were decalcified and were sectioned transversely or longitudinally at various thicknesses either on the freezing microtome or after embedding in paraffin. Serial sections were frequently made of paraffin embedded material.

Stains used included haematoxylin, either alone or with eosin; alum carmine; Masson's trichrome method; Weigert's resorcin fuchsin method for elastic fibres; Holmes's pyridine silver method (on the slide). A histological study was also made on a certain number of uninjected preparations.

Only that part of the nose anterior to the ethmoturbinals was examined in detail.

RESULTS

The arteries

In all the species of animals studied it was found that two main groups of vessels supply the nose: these are the sphenopalatine artery and its branches, and the ethmoidal group of vessels. In general, the former group supplies the respiratory portion of the nasal mucosa, while the latter group supplies the olfactory region.

The origin of these various vessels was determined during a study of the carotid rete and its associated arteries (Daniel, Dawes & Prichard, 1953). The sphenopalatine artery is a branch of the maxillary artery in the cat, dog, rabbit, goat, sheep and pig, but in the rat it arises from the infraorbital division of the stapedial artery. In man the ethmoidal vessels are supplied from the internal carotid system by means of the ophthalmic artery. In the above-mentioned animals, however, the ethmoidal group of arteries arises from a plexus in the olfactory fossa which has a double source of supply, namely, the external ethmoidal artery and the internal ethmoidal artery. In species other than the cat and the rat the external ethmoidal artery is a branch of the maxillary artery, and the internal ethmoidal artery arises from the circle of Willis. In the cat both the external and the internal ethmoidal arteries arise from the carotid rete. The right and left internal ethmoidal arteries join each other to form a single vessel which runs forward in the mid-line. This vessel, the nasal-meningeal artery, has additional roots on each side which spring from the arteria anastomotica or from the circle of Willis. The rat receives its ethmoidal supply from the internal carotid system, the external ethmoidal artery being a branch of the stapedial artery, while the internal ethmoidal artery is a branch of the circle of Willis. In all the species studied the olfactory fossa plexus was found to receive supplementary vessels from the branch of the anterior cerebral artery which supplies the olfactory bulb.

The course of the main arteries within the nose was found to be essentially similar in all the species studied, and resembles fairly closely that seen in man. The dog will be used primarily as a basis for description.

The sphenopalatine artery enters the nose through the sphenopalatine foramen, soon gives off a branch to the septum (the nasopalatine artery), and then continues along the lateral wall to the maxilloturbinal. Before reaching the maxilloturbinal this artery gives off several branches to supply the ethmoturbinal, the posterior part of the lateral wall of the nose, and the maxillary sinus. At the posterior end of the maxilloturbinal, the major vessel splits up into numerous branches, which run the length of the turbinal, and at the anterior end of the latter reform into a single vessel, which joins the infraorbital artery by a loop in the tip of the nose. The nasopalatine branch of the sphenopalatine artery crosses the roof of the nasopharynx below the sphenoid sinus, and runs along the wing of the vomer to reach the septum, where it continues in a forward and downward direction towards the nasopalatine canal, and there it anastomoses with the greater palatine artery. Close to the anterior end of the nasopalatine artery a branch turns upwards to join one of the long ethmoidal arteries. In the dog, cat, rabbit and rat the nasopalatine artery divides into several branches at a site opposite the posterior end of the maxilloturbinal.

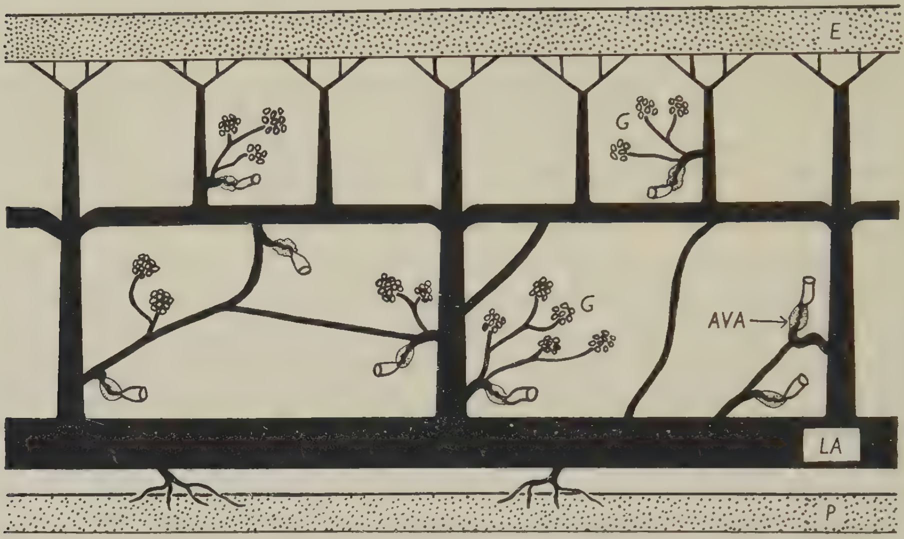
The ethmoidal group of vessels radiate forwards and downwards from the cribriform plate along the ethmoturbinals, the nasoturbinal and the corresponding region of the septum. The longest ethmoidal vessel in the lateral wall runs along the nasoturbinal close to its base as far as its anterior end, where it loops beneath the artery of the maxilloturbinal before joining it. The corresponding ethmoidal vessel running in the septum has a major communication with the nasopalatine branch of the sphenopalatine artery. Other long branches of the ethmoidal plexus run along the roof of the nasal cavity. With their free arterio-arterial anastomoses the branches of these arteries form two characteristic patterns. In the respiratory portion of the nasal mucosa, a latticework is formed which becomes a closer meshed net anteriorly (Pl. 1, fig. 1). This basic pattern holds good for both the septum and the lateral wall of the nose in the dog, cat, rabbit and rat, although it has to conform to the irregularities of the surface due to the turbinals. In the pig, goat and sheep the latticework arteries run obliquely in the septum (Pl. 1, fig. 2), whereas in the case of the maxilloturbinal the vessels run horizontally and much closer to one another (Pl. 1, fig. 3). In the olfactory mucosa the radial pattern is maintained and, although free anastomoses exist between the arteries, the major vessels lie close alongside one another and run along the ethmoturbinals.

The arteries forming these latticework patterns lie close to the periosteum (or perichondrium), and give off various branches. Some of these anastomose freely with one another, thereby forming an arterial arcade superficial to the major arteries. The height and disposition of the arcade varies according to the depth of the tissue in which it lies. The continuation of the trunks from which this arcade takes its origin, and some branches from the arcade itself run perpendicularly towards the surface, eventually dividing into several arterioles which spread out beneath the mucous membrane to supply the subepithelial capillary plexus (Text-fig. 1). The same vessels also supply the glandular and periosteal (or perichondrial) capillary plexuses. Throughout the depth of the tissue the arteries are connected by small anastomosing vessels.

Histologically, the large arteries within the nose have the typical thick muscular walls with an internal elastic lamina seen in arteries of a corresponding size elsewhere in the body. The medium and small-sized arteries appear to have relatively thin walls for arteries of their size, though in other respects they show the usual features.

The veins

The venous drainage of the nose is through large veins at its anterior and posterior extremities. Anteriorly, these veins communicate with the palatine veins below and with the anterior facial and the angular veins both below and above the lateral slit of the nostril. Valves are present in these veins where they join the venous plexus of the face. The veins draining the posterior part of the respiratory mucosa and the olfactory mucosa are collected into two main groups: (1) the ethmoidal veins, which pass through the cribriform plate and ultimately into the sagittal sinus, and (2) the sphenopalatine group of veins, which are continuous with the venous plexuses of the palate and nasopharynx, but are also collected into a large trunk which passes through the sphenopalatine foramen to enter the infraorbital vein.



Text-fig. 1. Schema of arterial pattern of nasal mucosa. The large vessel (*LA*) represents one of the 'latticework arteries'. The arterial arcades and the arteries which supply the epithelium (*E*), the glands (*G*), and the periosteum or perichondrium (*P*) are shown, and some of the sites at which arterio-venous anastomoses (*AVA*) have been found are indicated.

The most striking feature of the venous system within the nose is the great size and number of the veins compared with those of the neighbouring arteries. The detailed arrangement of the veins has as yet been worked out only in animals in which the veins lie superficial to the arteries (Swindle, 1935, 1937), i.e. dog, cat and rabbit. In these species there are two venous plexuses, both of which lie superficial to the arteries. The plexus situated nearest the surface is composed of small veins which form a fine network; the deeper plexus is formed by large veins and has a much coarser pattern. Structures which appear to correspond to the 'Ghost veins' reported by Swindle (1937) have occasionally been seen in this investigation but have been identified as nerves. When a well-filled Spalteholz preparation or a neoprene cast is viewed from the surface, one finds that the venous plexuses completely obscure the underlying arteries (Pl. 1, fig. 1). In the swell body the veins are connected by so many short communicating vessels that it is very difficult, even by dissection of a neoprene cast, to locate and trace the arteries. Veins from the deep venous plexus often loop around the arteries before anastomosing with other veins. The veins are intertwined, serpiginous in their course, as well as being in free communication with one another. In all preparations frequent junctions were seen between the superficial and deep plexuses, but the frequency and size of the communications seen between the large veins of the deep plexus, varied from specimen to specimen and from region to region. This difference appeared to be related to the extent to which the veins of this deep plexus were filled. Appearances similar to the 'venous swamps' described by Swindle (1937), and by others, have been seen in Spalteholz preparations, but have been attributed to unevenness of filling or to displacement of the injection mass when the mucosa was stripped from the bone. The 'beading' of veins described by Burnham (1935) has also been noted in neoprene casts.

The large veins of the deep plexus run roughly at right angles to the direction of the arteries, and when their anastomosing branches are well filled they form a latticework pattern, much more closely knit but otherwise similar to that of the arteries. These veins are present throughout the whole extent of the nasal mucosa but are especially numerous in the tip of the nasoturbinal, the swell body, the roof of the nose anteriorly, the upper part of the septum, and the septal tuberosity, where they communicate with one another very freely.

The venous system of the nasal mucosa is continuous with the venous system of the nasal tip, which in its turn drains into the angular and anterior facial veins. In the nasal tip the veins lie immediately under the skin and superficial to the arteries, and form a pattern quite different from that of the veins in the neighbouring hairy skin. Valves have been seen in veins in the nasal tip, but not in the venous system of the nasal mucosa.

On histological examination of the nasal mucosa, it was observed that in addition to thin-walled veins, such as those seen in other organs, there were numerous veins whose walls were unusually thick. These thick-walled veins were found throughout the nasal mucosa but were most commonly present in the 'erectile' or 'cavernous' tissues. They were identified as the vessels making up the deeper of the two venous plexuses. The thickness of the vessel wall was variable, and appeared to depend upon the degree of distension of the vessel. When the lumen was small the wall of the vessel was often very thick; when the vessel was greatly distended by the injection mass, the thickness of the wall was usually little if any greater than that normally seen in veins. Thick-walled veins were on the whole more frequently seen in uninjected specimens than in injected preparations.

Venules with thick walls have also been seen in addition to those of the usual type (Pl. 2, fig. 4). The thick-walled venules were found to drain the glandular tissue and open into thick-walled veins.

Some authors have found great difficulty in distinguishing between arteries and veins within the nose. The thick-walled veins are not modified arteries, as Swindle's work (1935, 1937) would suggest, and in serial sections they can be identified as veins because of their frequent connexions with other veins. Histologically, the distribution of muscle cells and the quantity of elastic fibres within their walls are different from those seen in an artery. The muscle cells of the venous wall have a much more irregular distribution than do those of an artery; this is also true of the thick-walled venules.

The capillaries

There are three main capillary plexuses, the subepithelial, the glandular, and the periosteal (or perichondrial).

(*a*) *Subepithelial capillaries.* Throughout the nose there is a close-meshed capillary network immediately beneath the epithelium. In the anterior part of the organ, where the epithelium is of stratified squamous type, loops arising from this subepithelial capillary plexus enter the papillae of the epithelium (Pl. 2, fig. 7). In the part of the nose lined by columnar ciliated mucosa no papillae exist and the capillary bed is limited to a flattened plexus which lies immediately beneath the basement membrane (Pl. 2, fig. 8).

The subepithelial capillary bed is supplied by small arteries arising from the 'latticework arteries' and from the arterial arcades described above. It is drained by venules which open into the superficial venous plexus.

(*b*) *Glandular capillaries.* This capillary bed is also supplied by arterioles arising from the 'latticework arteries'. The capillaries of several glands may be supplied by branches of the same arteriole. These glandular capillaries have a characteristic basket-like arrangement (Pl. 2, fig. 5). The arteriole enters the gland and within it breaks up into capillaries, which loop around the acini and then communicate with each other before draining into venules at the margin of the gland. The glandular ducts also have a capillary plexus which is wrapped around them. Because of the characteristic pattern of the glandular capillary plexus, the position of glands can be recognized in unstained injected preparations. The nasolacrimal duct is supplied by capillaries which form a plexus beneath the epithelium similar to the subepithelial plexus.

The blood from each gland is collected by several venules lying outside the gland (Pl. 2, fig. 5). These venules join with others draining adjacent glands to form a short vein which soon enters one of the veins of the deep plexus.

(*c*) *Periosteal* (*perichondrial*) *capillaries.* In the injected specimens very few capillaries were seen in the periosteum (or perichondrium). This observation, together with the fact that few arterioles or veins were seen supplying or draining this tissue, suggests that the latter has a poor blood supply. The periosteal capillaries receive their arterial supply from the same vessels as the subepithelial and glandular capillaries. They drain into veins which communicate with the bony plexus before joining the venous plexus of the tunica propria.

The capillaries in the nose show no unusual histological features.

Arterio-venous anastomoses

The term arterio-venous anastomosis is used here to describe a vessel by which blood can pass from an artery to a vein without traversing a capillary bed. This study has shown conclusively that such direct communications between arteries and veins are present in the nasal mucosa of the dog, the cat and the rabbit. Preparations of other species have not yet been examined for the purpose of determining whether arterio-venous anastomoses are present or not. The arterio-venous anastomoses which were found in the nasal mucosa closely resemble those seen in the tongue of the dog (Prichard & Daniel, 1953). We again apply the term arterio-venous anastomosis to the intermediate segment of the communicating vessel which is the only portion whose histological features differentiate it from other vessels. Injected preparations which demonstrate the arterio-venous anastomoses of the nose in their entirety have shown that although these vessels may vary in points of detail, they present certain characteristic morphological features. The arterio-venous anastomosis is frequently a tortuous vessel (Pl. 3, figs. 10, 12), its lumen is often small (Pl. 3, fig. 9) and has an irregular outline (Pl. 3, fig. 10). These vessels may be seen either singly or in groups of two to four lying close together, and occasionally the coils of two adjacent arterio-venous anastomoses are intertwined and form, as it were, a knot.

It has not been possible to determine the frequency and exact position of arterio-venous anastomoses in all parts of the nose, but the following observations regarding their distribution have been made. Numerous arterio-venous anastomoses are present in the olfactory region of the dog's nose. In this region their relationship to other structures has not yet been worked out. In the respiratory mucosa of the dog, cat and rabbit, arterio-venous anastomoses have been found in large numbers in the swell body, the nasoturbinal (Pl. 3, fig. 11), and in that portion of the septum (Pl. 3, fig. 9) which contains the greatest amount of vascular tissue and which is exposed to the main inspiratory airstream (Dawes, 1952*a*, *b*). They are also present in the maxilloturbinal, and in the tip of the nose (Pl. 4, fig. 13).

In the respiratory mucosa arterio-venous anastomoses usually arise from vessels of the arterial arcade or from the 'latticework arteries' (see Text-fig. 1). The short curving artery which gives rise to an arterio-venous anastomosis is often an early stout branch of an artery which eventually terminates in the subepithelial plexus. From the bend of the artery leading to the anastomosis, small vessels arise and can be recognized as those which supply the glandular tissue (Pl. 3, fig. 12).

In specimens where arterio-venous anastomoses have been readily seen, two features have not infrequently been observed. First, although the subepithelial capillary plexus has been well filled, the filling of the glandular capillaries has been slight. Secondly, the large veins of the deep venous plexus have been relatively empty. On several occasions an arterio-venous anastomosis was seen arising from each end of a small arterial arch and the latter, which supplied glands and also sent a few vessels to the subepithelial capillary plexus, had been reduced to a very small calibre. When an arterio-venous anastomosis is fully open the diameter of its lumen is at first similar to that of the artery from which it arises and gradually becomes greater as the vessel approaches the vein. Arterio-venous anastomoses open either directly into the thick-walled veins of the deep venous plexus, or into veins of the superficial venous plexus just before the latter communicate with the thick-walled veins.

Histologically, arterio-venous anastomoses in the nose of the cat and dog are characterized by their very thick walls, and the large number of cells which compose the media (Pl. 4, figs. 13, 14). Some of these are typical smooth muscle cells and others, having large pallid nuclei of oval or polyhedral shape, are of 'epithelioid' type (Pl. 4, fig. 15). No regular disposition of the different types of cells could be made out. The arterio-venous anastomosis has no internal elastic lamina. It was not possible to establish with certainty whether or not there was a continuous endothelial lining. In fixed preparations the lumen of the arterio-venous anastomosis is frequently small and irregular in outline. This irregularity is due to cells of the vessel wall projecting into the lumen. In histological preparations the diameter of the lumen was found to range from 8 to 30μ. In neoprene casts, however, in which the factor of shrinkage is absent, diameters ranging from 12 to 60μ were observed. In length arterio-venous anastomoses were commonly found to measure about $100–150\mu$.

DISCUSSION

In agreement with other workers who have studied the nasal angioarchitecture, we have been impressed by the great vascularity of the nasal mucosa and particularly by the great number and large capacity of the veins as compared with the arteries. These features suggest that a large quantity of blood can not only pass through the mucosa but can also be accommodated within it, mainly in its venous portion. It would also appear to be significant that in those structures which protrude into the nasal cavity such as the maxilloturbinal and nasoturbinal, the vessels as well as the glands are most numerous beneath the surface of that part of the mucosa which is most exposed to the inspiratory air stream (Pl. 2, fig. 6; Dawes, 1952*a*, *b*).

The present investigation has shown that arterio-venous anastomoses are definitely present in the nasal mucosa of the dog, the cat and the rabbit. Swindle (1935, 1937) did not believe that true arterio-venous anastomoses were present in the nose, but his description of what he termed 'pseudo-arterio-venous anastomoses' suggests that these vessels were in fact true arterio-venous anastomoses. Swindle's view that they were not true arterio-venous anastomoses was based on his belief that one end of the vessel opened into an atypical artery, whereas according to our findings the latter vessel is in fact a thick-walled vein. The structure which Swindle (1937, figs. 6, 7) observed in foetal material and termed a 'secondary arterio-venous anastomosis' in no way resembles a true arterio-venous anastomosis.

The arterio-venous anastomoses in the nasal mucosa show the same morphological and histological features as arterio-venous anastomoses situated elsewhere in the body (Hoyer, 1877; Grosser, 1902; Masson, 1937; Clara, 1939; Prichard & Daniel, 1953), and it is therefore not unreasonable to assume that they behave in a comparable manner. The observations of Grant (1930), Grant, Bland and Camp (1932) and Clark and Clark (1932, 1934) on arterio-venous anastomoses in the rabbit's ear during life showed that these vessels not only responded to various stimuli but also showed phasic variations in calibre.

The large number of arterio-venous anastomoses in the nose suggests that they must greatly influence the circulation through the nasal mucosa. Their situation, and the potential size of their lumen, indicate that when open, arterio-venous anastomoses will divert blood away from the glandular capillary plexus, and will also probably reduce the proportion of the total nasal blood flow received by the subepithelial plexus.

The variations in calibre which were seen in veins with thick muscular walls suggest that parts of the venous plexus can be shut down when not required to transmit blood. Alternatively, a sphincteric action of the large veins could obstruct the flow of blood out of the organ, and so permit the veins distal to this obstruction to be filled to their greatest capacity. Such a mechanism would explain the swelling of the mucosa which frequently occurs, but it would seem that the rapidity with which this swelling takes place must depend upon a sudden influx of blood into the veins, and this could be effectively accomplished by the opening of the arterio-venous anastomoses. The 'Drosselvenen' described by Körner (1937) in man have not been found in the nasal mucosa of animals.

The fact that arterio-venous anastomoses are present in the nasal mucosa, and the observation that some of the veins and venules have unusually thick muscular walls suggest that the blood flowing through the nose is subject to a mechanism which regulates not only its volume but also its regional distribution. Both the arterio-venous anastomoses and the thick-walled veins bear a special relationship to the capillaries of the glands or to the subepithelial capillary plexus. It would appear therefore that by their activity, either separately or possibly in conjunction with each other, these two types of vessel control the circulation through these capillary beds and thereby influence the functions of the nose. Fabbi & Rossatti (1951) appear to hold a similar view.

The respiratory portion of the nose carries out three main functions: warming, moistening and cleansing the inspired air. The warming of the inspired air is achieved by the air streams taking up heat from the walls of the nasal cavity. The main source of heat is presumably provided by the blood in the superficial vascular system, in particular by the subepithelial capillary plexus. Changes of blood flow through the superficial system will alter the amount of heat available for warming the inspired air. Basic changes in the flow through the subepithelial capillary plexus have been reported by Sternberg (1927). Whether or not circulatory changes which affect heating of the air are caused by activity of arterio-venous anastomoses remains an open question, but in any case Perwitschky's (1927) findings suggest that heating of the inspired air is a less important function of the nose than moistening of the inspired air.

The means by which the inspired air is moistened and cleansed are closely related. The secretion of the glands forms the mucous blanket which covers the mucosal surface and thereby provides both moisture for humidification of the inspired air and also a continuous absorbent sheet which can be moved by the cilia for cleansing purposes. The degree of viscosity of the mucous blanket, which is of great importance to the effectiveness of ciliary action (Proetz, 1941), is affected not only by the loss of fluid into the inspired air but also by the amount and type of glandular secretion. Changes in the course of the circulation which affect the amount of blood passing through glandular capillaries, would in themselves therefore influence indirectly both the humidity of the inspired air and the cleansing of the nasal cavity. The findings in this investigation have indicated that the circulation through the glands is probably regulated by the activity of the arterio-venous anastomoses.

Whatever the most important of the respiratory functions of the nose, each of them must be greatly influenced by the circulation through the organ. The constantly varying atmospheric and other conditions demand frequent adjustments of the circulation to meet the needs of the moment. The present work has suggested that effective mechanisms for making such adjustments are provided by the arterio-venous anastomoses and the thick-walled veins which are present in the nasal mucosa.

SUMMARY

The vascular arrangements of the nose of the dog have been studied by means of injection and histological methods. Observations have also been made on several other species of animal.

The distribution of the arteries and veins of the nasal mucosa and the patterns formed by these vessels and by the intervening capillary plexuses are described. A feature of particular interest is the presence of veins and venules with unusually thick muscular walls.

Arterio-venous anastomoses have been found in the nasal mucosa of the dog, cat and rabbit. Some features of their morphology, histology and distribution are described. The arterio-venous anastomoses appear to be related chiefly to the glandular capillary beds and to open into the thick-walled veins.

The possible bearing of these findings on the physiology of the nose is briefly discussed.

We are grateful to Mr J. W. Milligan for help with the histological preparations.

REFERENCES

Bachi, S. (1947). Particolarità strutturali di piccole arterie della mucosa dei cornetti medio e inferiore. *Arch. ital. Otol.* **58**, 161–165.

Burnham, H. H. (1935). An anatomical investigation of blood vessels of the lateral nasal wall and their relation to turbinates and sinuses. *J. Laryng.* **50**, 569–593.

Clara, M. (1939). *Die arterio-venösen Anastomosen.* Leipzig: J. A. Barth Verlag.

Clark, E. R. & Clark, E. L. (1932). Observations on living preformed blood vessels as seen in a transparent chamber inserted into the rabbit's ear. *Amer. J. Anat.* **49**, 441–477.

Clark, E. R. & Clark, E. L. (1934). Observations on living arterio-venous anastomoses as seen in transparent chambers introduced into the rabbit's ear. *Amer. J. Anat.* **54**, 229–286.

Daniel, P. M., Dawes, J. D. K. & Prichard, Marjorie M. L. (1953). Studies of the carotid rete and its associated arteries. *Philos. Trans.* B, **237**, 173–208.

Dawes, J. D. K. (1952*a*). The vascular arrangements of the nose and their significance in relation to the nasal airways and airstreams. Thesis for B.Sc. Degree, University of Oxford.

Dawes, J. D. K. (1952*b*). The course of the nasal airstreams. *J. Laryng.* **66**, 583–593.

Fabbi, F. & Rossatti, B. (1951). Sulla presenza di anastomosi artero-venose e di dispositivi di blocco nel turbinato inferiore dell'uomo. *Oto-rino-laryng. ital.* **19**, 305–318.

Grant, R. T. (1930). Observations on direct communications between arteries and veins in the rabbit's ear. *Heart*, **15**, 281–303.

Grant, R. T., Bland, E. F. & Camp, P. D. (1932). Observations on the vessels and nerves of the rabbit's ear with special reference to the reaction to cold. *Heart*, **16**, 69–101.

Grosser, O. (1902). Ueber arterio-venöse Anastomosen an den Extremitätenenden beim Menschen und den krallentragenden Säugethieren. *Arch. mikr. Anat.* **60**, 191–216.

Guaragna, C. (1940). Osservazioni sulla struttura dei vasi della mucosa nasale della regione respiratoria. *Arch. ital. Otol.* **52**, 615–638.

Harper, W. F. (1949). Further observations on the blood vessels of the nasal mucous membrane in mammals. *J. Anat., Lond.*, **83**, 61.

Hoyer, H. (1877). Ueber unmittelbare Einmundung kleinster Arterien in Gefässäste venösen Charakters. *Arch. mikr. Anat.* **13**, 603–644.

Kohlrausch, O. (1853). Ueber das Schwellgewebe an den Muscheln der Nasenschleimhaut. *Arch. Anat. Physiol., Lpz.*, pp. 149–150.

Körner, F. (1937). Über Drosselvenen im Schwellgewebe der Nasenschleimhaut. *Z. mikr.-anat. Forsch.* **41**, 131–150.

Märk, W. (1941). Über arterio-venöse Anastomosen, Gefässsperren und Gefässe mit epitheloiden Zellen beim Menschen. *Z. mikr.-anat. Forsch.* **50**, 392–445.

Masson, P. (1937). *Les glomus neuro-vasculaires.* Paris: Hermann et Cie.

Perwitschky, R. (1927). Die Temperatur und Feuchtigkeitsverhältnisse der Atemluft in den Luftwegen. *Arch. Ohr.-, Nas.-, u. KehlkHeilk.* **117**, 1–36.

Prichard, Marjorie M. L. & Daniel, P. M. (1953). Arterio-venous anastomoses in the tongue of the dog. *J. Anat., Lond.*, **87**, 66–74.

Proetz, A. W. (1941). *Essays on the Applied Physiology of the Nose.* St Louis: Annals Publishing Co.

Sternberg, H. (1927). Ueber die Blutverteilung, resp. Capillarinjektion in der Schleimhaut der Luftwege und ihre physiologische und pathologische Bedeutung. *Z. Hals- Nas.- u. Ohrenheilk.* **18**, 593–598.

Sucquet, J. P. (1862). *D'une circulation dérivative dans les membres et dans la tête chez l'homme.* Paris: Adrien Delahaye.

Swindle, P. F. (1935). The architecture of the blood vascular networks in the erectile and secretory lining of the nasal passages. *Ann. Otol., etc., St Louis*, **44**, 913–932.

Swindle, P. F. (1937). Nasal blood vessels which serve as arteries in some mammals and as veins in some others. *Ann. Otol., etc., St Louis*, **46**, 600–628.

Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J. & Prichard, M. M. L. (1947). *Studies of the Renal Circulation.* Oxford: Blackwell Scientific Publications.

Zuckerkandl, E. (1885). Über den Circulations-Apparat in der Nasenschleimhaut. *Denkschr. Akad. Wiss. Wien*, **49**, Abt. ii, 121–152.

EXPLANATION OF PLATES

Plate 1

Fig. 1. Cat. Neoprene cast of vessels of right side of nasal septum seen from lateral aspect. Note the latticework pattern of the arteries, and anteriorly (on the right) the well-filled venous plexus lying superficial to the arteries. (× 9.)

Fig. 2. Pig. Neoprene cast of vessels of septum showing oblique disposition of the arteries. (× 3·1.)

Fig. 3. Sheep. Neoprene cast of vessels of maxilloturbinal. Note that the arteries run horizontally and are closely packed together. (× 3·3.)

Plate 2

Fig. 4. Maxilloturbinal of dog (injected preparation). A venule is seen to left of centre. Note the thick wall of this vessel as compared with the walls of the arterial vessels (grey injection mass) and of the vein (black injection mass). (Paraffin section, haematoxylin and eosin; × 230.)

Fig. 5. Swell body of dog (injected preparation). To show basket-like pattern and venous drainage of glandular capillary plexus. (Thick frozen section; × 72.)

Fig. 6. Transverse section of nasoturbinal of dog (injected preparation). The greater number of vessels and glands are situated on the medial surface (right). This is the surface which is exposed to the main inspiratory airstream (Dawes, 1952*a*, *b*). (Paraffin section, haematoxylin and eosin; × 28.)

Fig. 7. Mucosal vessels in swell body of dog (injected preparation). Note the capillary loops projecting into the epithelium. This is a characteristic feature of this area which is covered by stratified squamous epithelium. (Thick frozen section; × 88.)

Fig. 8. Mucosal vessels in lateral wall of dog's nose near maxilloturbinal (injected preparation). In this region, where the epithelium is of columnar ciliated type, the subepithelial capillary plexus is flattened and lies entirely beneath the basement membrane. (Thick frozen section; ×88.)

PLATE 3

Fig. 9. Spalteholz preparation of nasal septum of dog (injected). Three arterio-venous anastomoses are indicated by arrows. Note the narrow lumen. *A*, artery; *V*, vein. (×70.)

Fig. 10. Frozen section of swell body of dog injected with Berlin blue. An arterio-venous anastomosis is seen arising from an artery and draining into a vein (upper right). Note the tortuous course of the anastomosis. The cells of the wall of the anastomosis are outlined by the injection mass. (×220.)

Fig. 11. Neoprene cast of vessels of nasoturbinal of dog showing a widely open arterio-venous anastomosis (at arrow). *A*, artery; *V*, vein. (×33.)

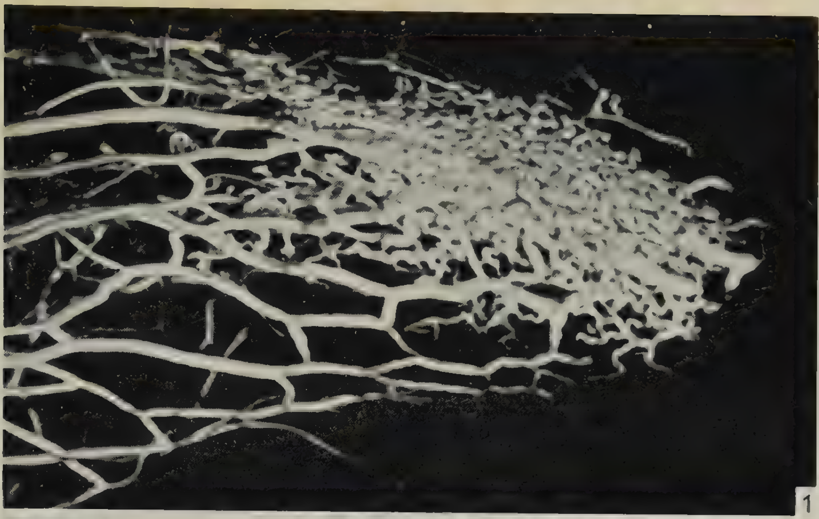
Fig. 12. Frozen section of swell body of dog injected with Berlin blue. The large vessel is one of the 'latticework arteries' and is seen giving rise to an arterial arcade. The artery springing from the left side of the arcade supplies a gland and also an arterio-venous anastomosis (at arrow). (×28.)

PLATE 4

Fig. 13. Tip of dog's nose (injected preparation). An arterio-venous anastomosis is seen (at arrow) draining into a vein (*V*). The artery from which it arises can be seen above (*A*). Note the tortuous course and the thick wall of the anastomosis. (Frozen section, alum carmine; ×132.)

Fig. 14. Septum of dog (injected preparation). One arterio-venous anastomosis is seen (at arrow on left) connecting an artery (*A*) with a vein (*V*). Part of a second arterio-venous anastomosis is also seen (at arrow on right) arising from the same artery; this anastomosis was found to drain into a vein in an adjacent section. Note the thick cellular walls of these anastomotic vessels. (Paraffin section, haematoxylin; ×220.)

Fig. 15. Arterio-venous anastomosis in swell body of dog (injected preparation). The vessel is cut somewhat obliquely. Note the large pallid nuclei of the 'epithelioid' cells in the thick wall of the vessel and the narrow irregular lumen. (Paraffin section, Weigert's elastic stain and haematoxylin; ×710.)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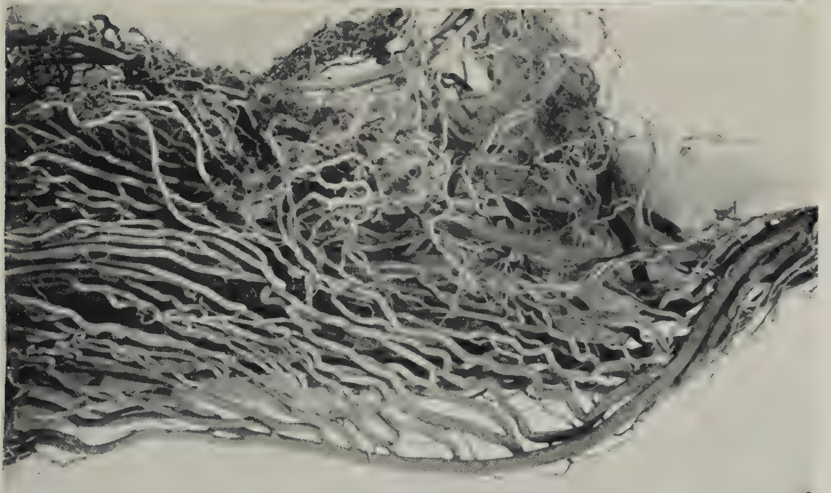
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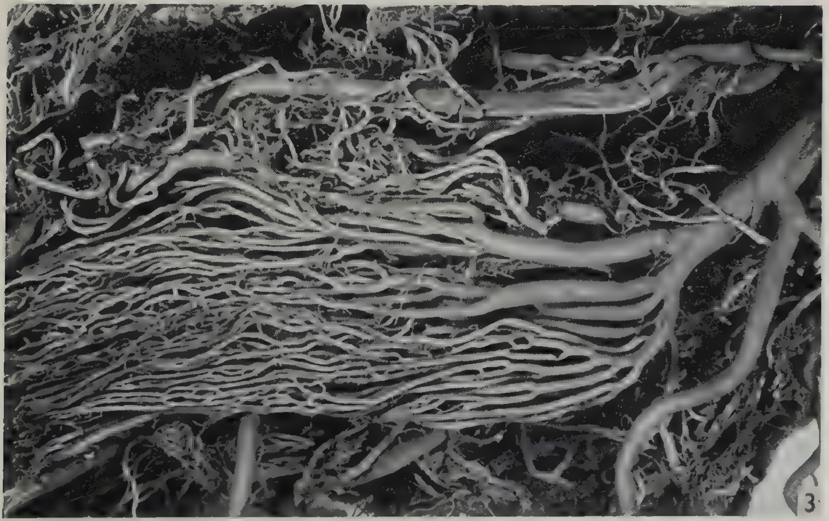
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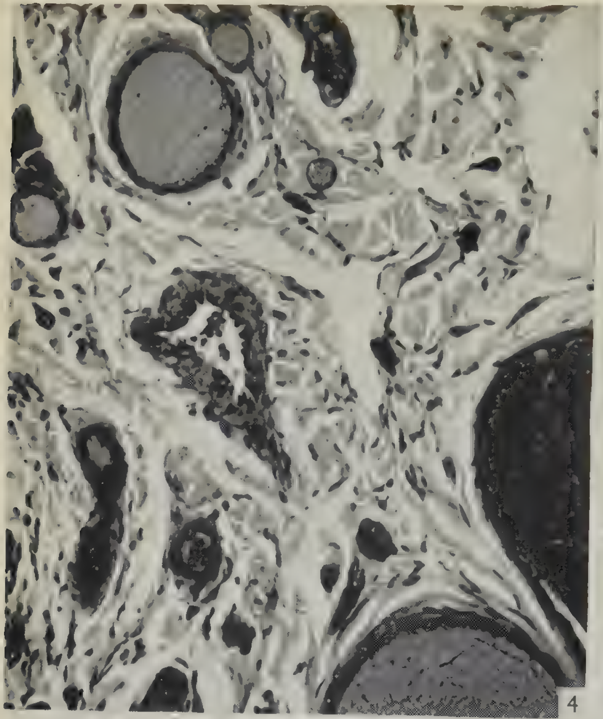
DAWES AND PRICHARD—STUDIES OF THE VASCULAR ARRANGEMENTS OF THE NOSE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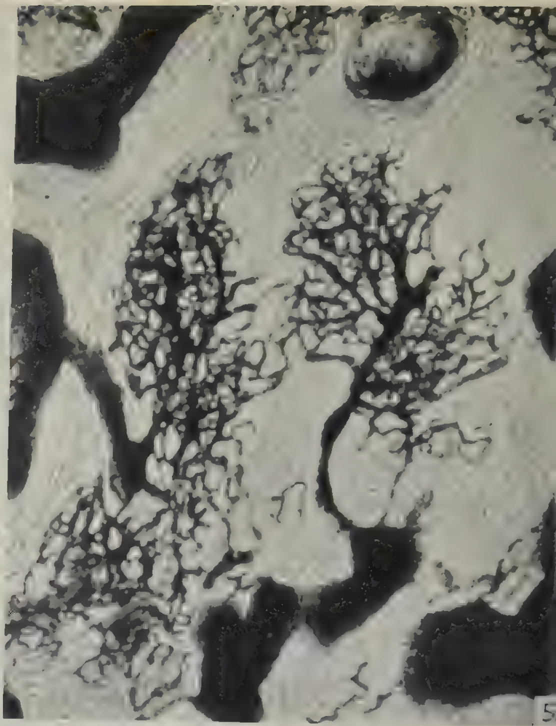

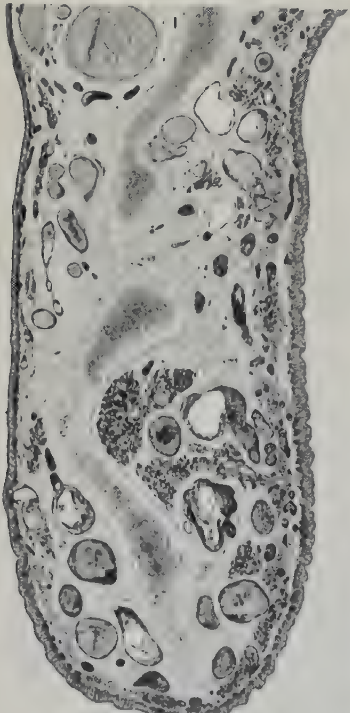
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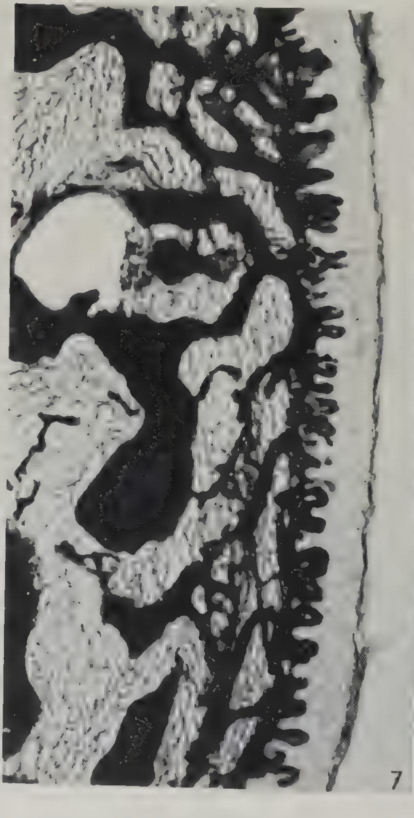
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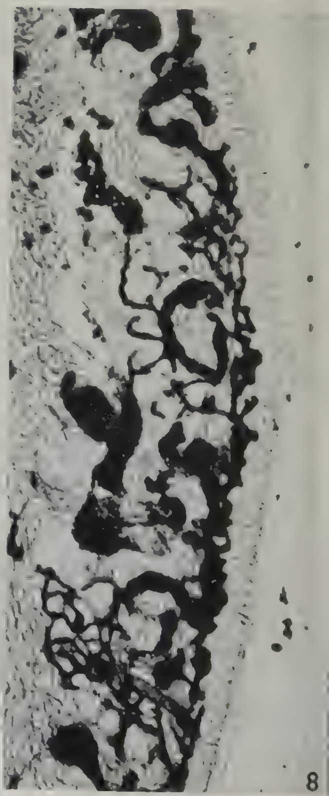
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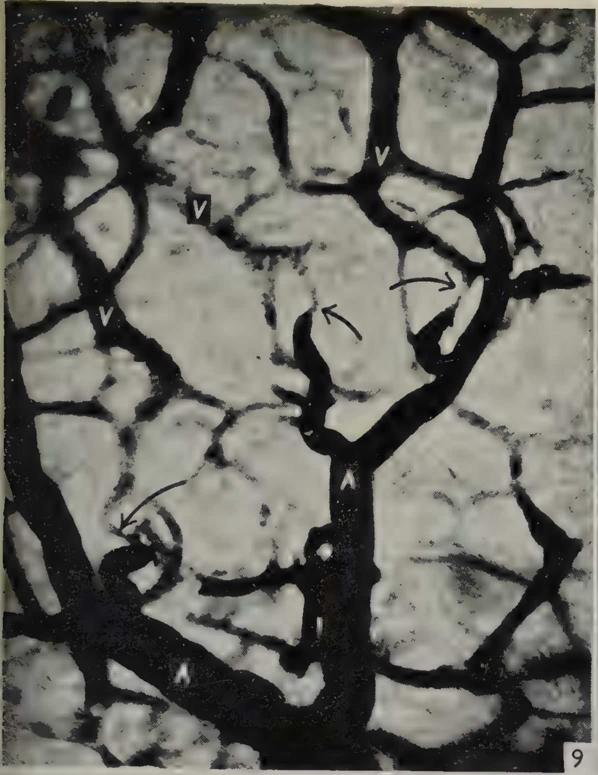

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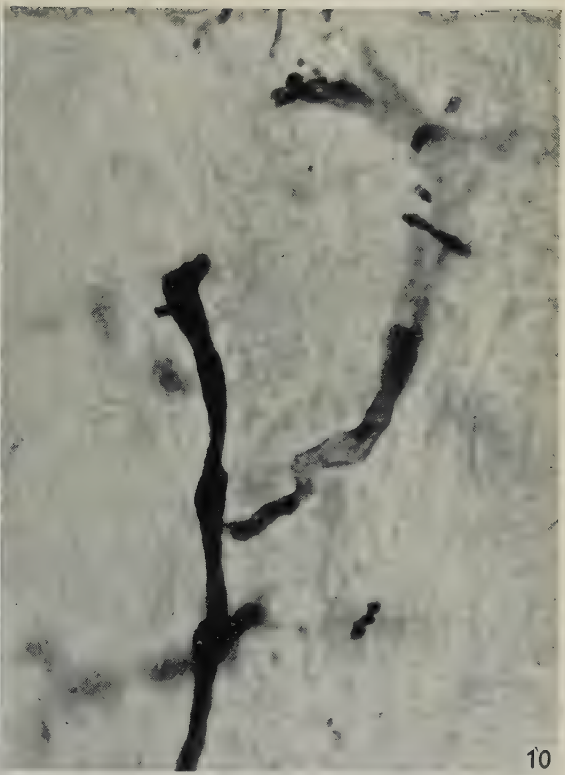

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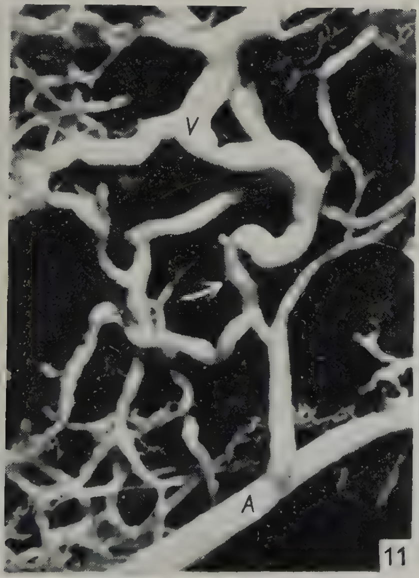

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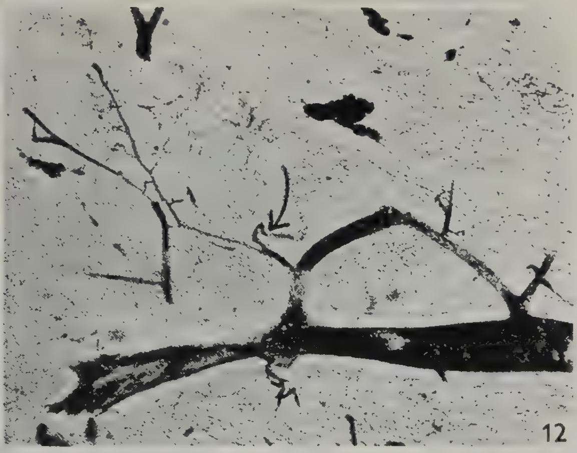

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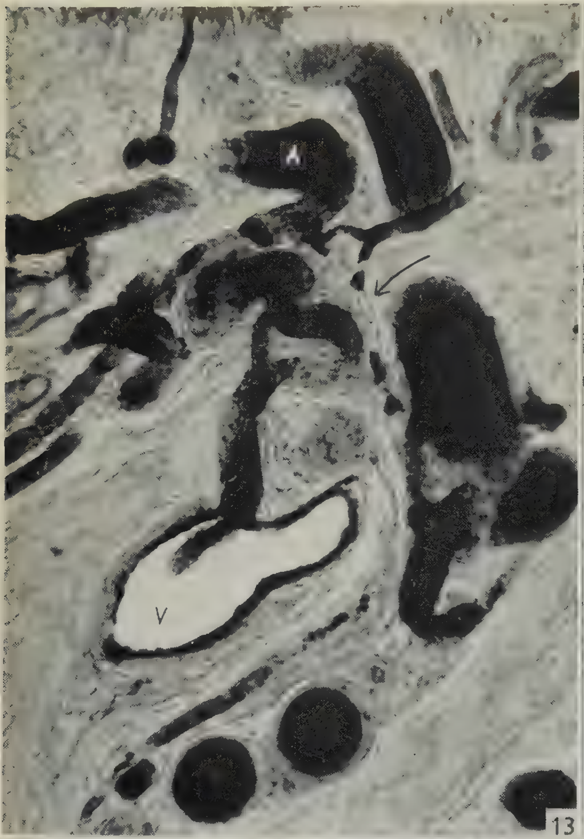

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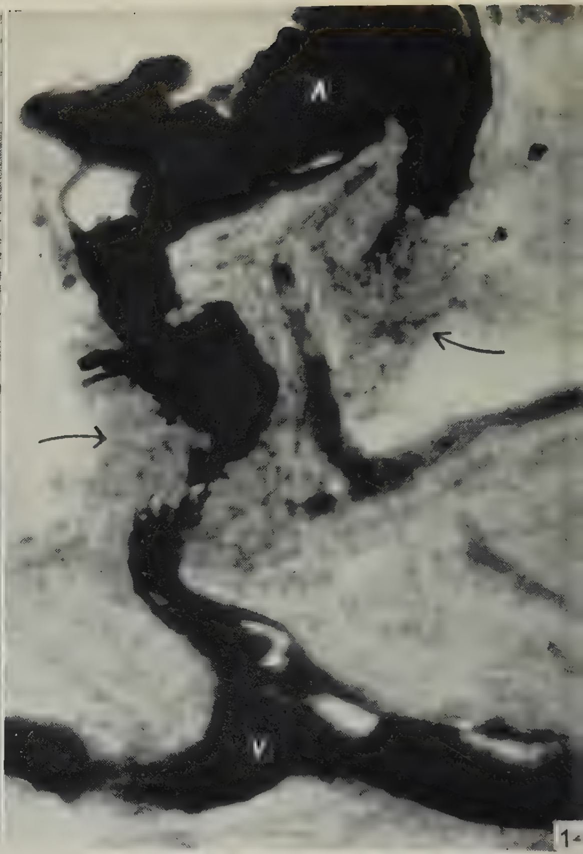

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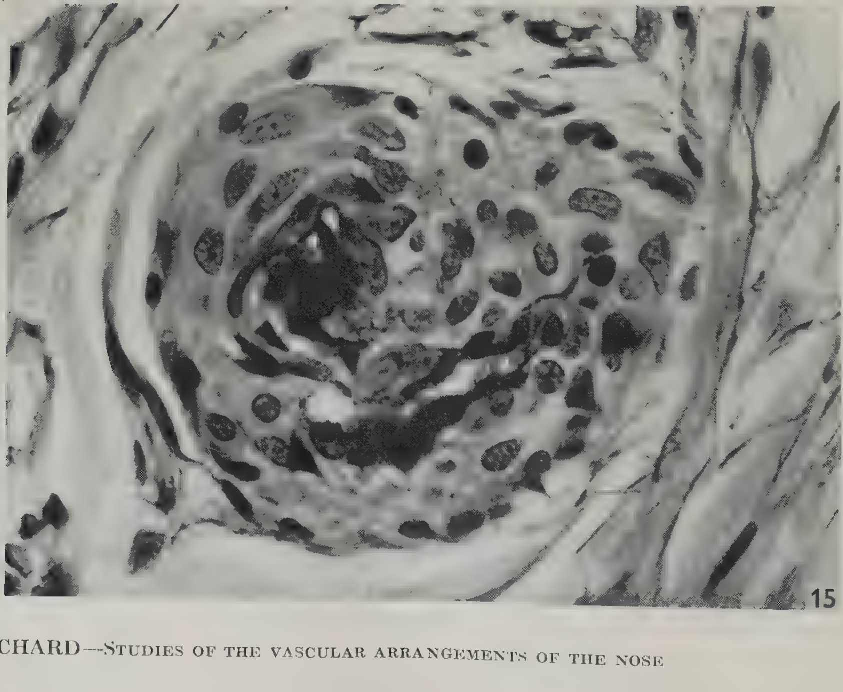

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DAWES AND PRICHARD—STUDIES OF THE VASCULAR ARRANGEMENTS OF THE NOSE

IN MEMORIAM

W. K. CONNELL, M.B., F.R.C.S. (Eng.), F.R.F.P.S.G., 1894–1952

William Kerr Connell, lecturer in Anatomy in the University of Glasgow, died on 31 October 1952 at the age of 58. He was educated at Glasgow Academy and the University of Glasgow. Connell interrupted his medical studies to serve as a private in the 9th H.L.I. from September 1914 till March 1915. He was then released from the Army to complete his medical studies. In 1916 having graduated M.B., Ch.B., he joined the R.A.M.C. and served until 1920 retiring with the rank of Captain. For a time he was surgical specialist with the Derajat Brigade. Following his demobilization Connell was for a time house surgeon to the late Prof. Archibald Young in Glasgow and to Sir Hugh Lett of the London Hospital.

From 1921 to 1922 he was a demonstrator of Anatomy in the University of Glasgow under the late Prof. T. H. Bryce. In 1924 Connell joined in the Colonial Medical Service and, until 1946, was mainly engaged as a surgical specialist in Tanganyika Territory. During this period he was also active in teaching the principles of anatomy and surgery to the English-speaking African Hospital Assistants. In 1927 Connell became a Fellow of the Royal College of Surgeons of England, and three years later he took the D.T.M. & H.

During the recent World War Connell was seconded from the Colonial Medical Service to the Army. He held the rank of Lieutenant-Colonel and for his services was mentioned in Dispatches.

In July 1946 Connell was appointed to succeed Prof. John Graham in the Chair of Anatomy at the Anderson College of Medicine, Glasgow. In his new sphere of activity he worked with great enthusiasm. When Anderson College was absorbed into Glasgow University, Connell was appointed to a Lectureship in the Department of Anatomy. He had acquired by now an extensive knowledge of topographical anatomy and taught with great keenness. While interested in research he realized he had no particular flair for experimental investigations. He was not, however, lacking in original ideas. He was the author of a Surgical Handbook which was extensively used by the English-speaking African Assistants. To his widow we offer our sincerest sympathy.

W. J. H.

OBITUARY NOTE

We regret to announce the untimely death of Chalmers Bruce Murray, Ph.D., B.Sc., M.R.C.V.S., on 23 January 1952. He was lecturer in Histology at the Royal Veterinary College.

REVIEWS

Histologie und mikroskopische Anatomie des Menschen. By WOLFGANG BARGMANN. Vol. I. *Zellen- und Gewebelehre.* (Pp. viii + 210; 193 illustrations; 10½ × 7 in.; DM. 19.60.) Stuttgart: Georg Thieme Verlag. 1948. Vol. II, *Organe und Systeme.* (Pp. xii + 563; 412 illustrations; 10½ × 7 in.; DM. 45.) Stuttgart: Georg Thieme Verlag. 1951.

Recent times have seen the publication of a number of new text-books of histology; this is not surprising in view of the new impetus which this subject has received due, on the one hand, to the introduction of a variety of new techniques, physical and chemical, which have been and are being applied to cytological and histological problems, and on the other to a more functional and dynamic approach. The latter has been the mainspring of Prof. Bargmann's magnum opus. Consequently, 'πάντα ῥεῖ' is written as a motto on one of its pages, and a forceful dynamic style of writing seems to emphasize the underlying outlook. One does not usually look for simplicity of expression in a German text-book, yet here the language flows on the whole well and evenly, only very occasionally breaking on one of the familiar rocks of an unnecessarily involved, intractable construction. In general, the work claims to be a text for students—and reference is made, here and there, to beginners—yet with reading the impression is gained that it is addressed rather to the initiated or even the expert.

The first volume (cyto- and histology) still bears the mark of the aftermath of war. The quality of the paper is not very good and some of the illustrations, especially microphotographs, have suffered in reproduction. Contact with the world outside Germany had probably not yet been established, and so almost all the recent references are to German publications. This has been remedied to some extent in the second volume in which some more recent work from the world literature is cited, but often authors are mentioned in the text who do not appear in the list of references, and vice versa.

Vol. I opens with a brief historical survey, followed by a chapter on methods of investigations; completeness is aimed at, but extreme condensation makes it difficult to follow this account. Next, the living matter and its ultrastructure is dealt with in an interesting manner with detailed mention of modern physical and chemical methods. The chapter on epithelia lacks clarity and precision; the description of nerve fibres is confusing, and undue emphasis is given to the still problematic terminal nerve network and the syncytial nature of nervous tissue in general. The account of the connective tissues is very comprehensive and penetrating, but one must question, at least from the didactic point of view, the wisdom of interspersing dentine formation—quite abruptly—in the midst of a dynamic description of bone development.

Vol. II (organs and systems) is beautifully produced. The numerous illustrations, mainly line drawings, are first class; their value would be further enhanced by some more detailed annotations. Many depict the organs at hand-lens magnification, a special and most successful feature of the book. The borderland between macroscopic and microscopic anatomy—a much neglected level—is thus brought into focus. In keeping with this tendency long passages are devoted to the 'functional adaptation' of the mechanical tissues. In fact, the all-pervading connective tissue gets more than its fair share, whilst the description of the actual parenchyma is sometimes given almost in parenthesis.

Of the structure-function relationships the more problematic ones hold the field, whilst the known and well-founded ones (effects of hormones, vitamins and other chemicals)—though not omitted—are not sufficiently stressed or elaborated; the maturation factor for red blood cells is not even mentioned.

The main quarrel which the reviewer has with the author is over the mass of dogmatic or near-dogmatic statements which one has to suffer almost on every page. It would be euphemism to call them hypotheses, as the author implies in the preface. Many of these

—too long to be cited—concern functional attributes to structures; in addition, one reads that every liver cell is innervated; pericytes are outside the reticulum of the liver sinusoids; the stainable matter of reticulocytes is due to nuclear material; placental septa are made of trophoblastic cells; fat cells are connected by fine protoplasmic processes; the macrophage is a functional stage of the fibrocyte; Langerhans' cells of the epidermis and binucleate ganglion cells of the autonomic system are degenerate forms; amitosis plays a large part in the regeneration of various tissues; and the formation of binucleate cells seems *a priori* due to this process. These are but a few examples. Often such pronouncements are unobtrusively qualified by adverbs which range from 'clearly, obviously, doubtless' over 'probably, possibly' to 'perhaps' with many intermediate shades.

The abundance of such statements mars an otherwise interesting and unique text which in places achieves a high degree of perfection in presentation (e.g. veins, nasal mucous membrane, trachea, gall bladder, above all mammary gland—to mention only a few). The overall compass of the work is exceptionally wide, ranging as it does from the level of dissection to X-ray diffraction patterns of matter with functional contemplations at every level, and also including embryological information, however much some of its startling ideas and statements may challenge histologists.

Not so long ago a new text-book of Histology published in the English-speaking world was reviewed in these pages, a book also written with a strong physiological outlook. No greater contrast between two works of this kind can easily be imagined. Is this due more to 'nature or nurture', one wonders? F. JACOBY

Studies on the Morphogenesis of the Brain in Birds. (*Morphogenesis of the Vertebrate Brain. V.*) By K. J. KRABBE. (Pp. 101; 53 plates; 12 × 9 in.; £2. 10*s.*) Copenhagen: Epnar Munksgaard. 1952.

This is the fifth monograph on the morphogenesis of the vertebrate brain produced by Prof. Krabbe. Like the previous volumes, which dealt with reptilian and mammalian brains, it is based on an examination of serial sections of embryos from the collection in the Tornblad Institute of Comparative Embryology in Lund.

Developmental stages are described for fifteen birds, including such widely different species as an emu, an owl, humming bird, swan, penguin, etc. The stages range from about 5 to 50 mm. in length; the youngest have a completely closed neural tube, with the main subdivisions of the brain clearly marked; in the oldest, the adult form of the brain has been acquired, and the differentiation of nuclei and cellular laminae is well advanced. All the descriptions are based on wax-plate reconstructions of the brain and are fully illustrated. For most species several stages are described.

The descriptions deal almost exclusively with the form and relative size of the main subdivisions of the brain so far as these can be studied in reconstructions. The cellular differentiation of such structures as the corpus striatum, the cerebral cortex or the tectum of the midbrain, while occasionally mentioned, is not described in any detail. Some photographs of actual sections are reproduced, but the magnification is too low to show much of the histological structure of the parts of the brain they illustrate. The majority of the illustrations are drawings of the reconstructions, and of course show no more than the external features of the brain.

By providing standard illustrations of the form of the brain at known stages in the development of a variety of different birds this monograph will have considerable value to anyone beginning work on the more detailed morphogenesis of the brain in a particular species. It also has obvious value as a record of observations, but does not add much to our theoretical knowledge of the morphogenesis of the brain in general, nor to knowledge of the systematic position of birds, either in comparison with other vertebrate classes, or among themselves. The short last chapter dealing with these matters and with certain functional considerations gives little that could not be deduced from an examination of the morphology of the adult brain in the different species.

There is a short historical introduction, and a full bibliography; apart from the list of contents at the beginning, no index is provided. F. GOLDBY

Das Inselsystem des Pankreas. By Helmut Ferner. (186 pp., 54 abb. Gänzl. DM. 29.70.) Stuttgart: Georg Thieme. 1952.

This book is a comprehensive account of the pancreatic islets. It deals with their development, their microscopical structure and their histo- and patho-physiology. There is a detailed section on modern staining methods for differentiating the α and β cells, and the author discusses the significance of the results obtained from their use. This is followed by sections on the development of the islet cells in man and on their histological characteristics. The author also discusses the histochemistry of the islets including distribution of vitamin C, alkaline phosphatase, neutral fat, lipoid, acetalphosphatide and fluorescent substances.

There is also a section on experimental morphological studies of the islets in seven species of mammals and the final chapter deals with the changes in the islets in diabetes mellitus.

There are 54 figures, most of them excellent photomicrographs and a literature list containing about 240 references.

In general one can say that this book is an up-to-date, detailed and well-documented account of the islet cells. There is only one obvious serious fault, and that is the absence of an index.

GEOFFREY H. BOURNE

THE CARBOHYDRATE COMPONENTS OF THE VAGINA OF THE NORMAL AND OVARIECTOMIZED MOUSE DURING OESTROGENIC STIMULATION

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The earliest description of the histology of the vagina of the mouse is that of Morau (1889), who was concerned mainly with changes occurring in the vaginal epithelium at the beginning of pregnancy. Since the sexual cycles of animals had not been recognized at that time no correlation was noted between the appearance of the vaginal epithelium and the stages of the oestrous cycle. Morau noted, however, that cornification occurred at the time when mating normally takes place (i.e. oestrus); that the epithelium reverts to a low stratified form if pregnancy does not ensue, and that five days later the cornification reappears. Several other French histologists made similar observations with rodents but were not specifically concerned with the mouse.

The first important paper on the vaginal epithelium of the mouse is that of Allen (1922). Prior to this, Stockard & Papanicolaou (1917) showed that in the guinea-pig the vaginal epithelium undergoes cyclical morphological changes which may be correlated with the phases of the ovarian cycle, and that these stages may be easily diagnosed by the microscopical examination of vaginal smears. Also, in 1922, Long & Evans showed that similar changes occur in the vaginal epithelium and smears of the rat.

Allen & Doisy (1923) and Allen, Francis, Robertson, Colgate, Johnston, Doisy, Kountz & Gibson (1924) described the effect of double ovariectomy in the mouse. They showed that 4–6 days after the operation the vaginal epithelium is in the resting (dioestrous) state consisting of from two to four layers of cells. This state persists for about 6 weeks, after which slow progressive atrophy occurs. However, they noted that even after 15 weeks the vaginal epithelium will respond to an injection of oestrogen. Following a subcutaneous injection of oestrogen the basal cells of the stratum germinativum proliferate, and at 36 hr. after an injection there are 12–15 layers of cells. An outer 'vacuolar' layer represents the remnants of the resting vaginal epithelium, and beneath it cornification occurs by transformation of the stratum granulosum. Although cornification occurring below the superficial layer of cells was first described by Retterer (1892) in the guinea-pig, it was Long & Evans (1922) who drew attention to the singular position in which it occurs in the epithelium. The cornified and superficial layers are subsequently sloughed off and are destroyed together with the outer layers of the epithelium by a heavy infiltration of leucocytes.

Many workers have shown that the local application of oestrogens to the vagina results in cornification of the epithelium. The earliest study is that of Loewe & Voss (1926) in the guinea-pig, and since then many workers have confirmed the effect in both the rat and mouse (see Emmens (1950), for a review of the literature). However, histological studies in the rat (Freud, 1939) and in the mouse (Biggers, 1952) have failed to detect any differences between the cornified epithelium produced by intravaginal or subcutaneous administration of oestrogen. The only difference detected in the mouse is that the average time of onset of cornification is slightly less with the intravaginal method.

Few detailed histochemical studies have been made of the vagina. In the mouse the only work seems to be that of Jeener (1948) and Kamell & Atkinson (1948), who followed the changes in alkaline phosphatase, desoxyribonucleic acid, and ribonucleic acid during the process of cornification. Studies of the carbohydrate components are lacking, except in the monkey and in woman where cyclical changes in the glycogen content of the vagina during the menstrual cycle and pregnancy have been examined (for the literature see Lison & Vokaer, 1949; and Foraker & Brawner, 1951). In order to gain further insight into the cellular processes which occur in the cornifying vaginal epithelium of mice, the changes in the carbohydrate components have been studied both in ovariectomized animals under the influence of oestrone and in intact mice undergoing their normal cycles.

MATERIALS AND METHODS

Forty ovariectomized mice have been used in this study. Twenty were given 0·5 μg. oestrone subcutaneously in nut oil, and the other twenty 0·002 μg. oestrone intravaginally in water. The oestrogen solutions were prepared according to methods described elsewhere (Biggers, 1951). In both cases the oestrogen was injected in two doses, separated by an interval of 24 hr., the total dose being large enough to ensure cornification in all animals. At intervals, groups of mice were killed and the vaginae prepared for histological examination. Since the time of onset of cornification is independent of dose, provided the doses are maximal (Biggers, 1952), the results obtained by both routes of administration should be comparable.

In addition, eight intact mice were examined to see if the changes which occur naturally are similar to those produced by artificial oestrogenic stimulation. Pairs were killed at each of the four main stages of the oestrous cycle as judged by the vaginal smear. Daily examination of the vaginal smears for some days before the mice were used showed that they were undergoing normal oestrous cycles.

The vaginae were fixed in either Zenker-formol, picric-alcohol or 4 % basic lead acetate followed by neutral buffered formalin. Sections were cut at 5–7 μ.

The sections were stained with (i) haematoxylin and eosin, (ii) the Lillie Allochrome method (Lillie, 1951*a*), (iii) the periodic acid Schiff (PAS) technique (McManus, 1946) and (iv) 0·5 % toluidine blue.

RESULTS

Histological and cytological aspects of the vaginal response

The histological changes in the vaginal epithelium of ovariectomized mice following the systemic and local administration of oestrone are illustrated in Pl. 1. No difference can be seen in the response for either method of administration. In both cases keratinization is seen to occur beneath a superficial layer of cells representing the former vaginal epithelium.

The resting epithelium is two to three cells thick, the cells being small with densely staining nuclei (Pl. 1, fig. 1), the basal cells forming the stratum germinativum. Following the administration of oestrone the cells of the stratum germinativum divide and the daughter cells migrate outwards pushing the old superficial cells before them and giving rise to a new layer of cells having distinct cell outlines, a large amount of cytoplasm, and large vesicular nuclei with up to three prominent nucleoli (Pl. 1, figs. 2, 3). These daughter cells appear to have intercellular bridges between them and collectively constitute the stratum spinosum (or prickle cell layer). As cornification proceeds they continue to move outwards, and in so doing become flattened and develop granular material in the cytoplasm and form the stratum granulosum. Peripheral to the stratum granulosum, fully cornified cells are found, comprising the stratum corneum (Pl. 1, figs. 4, 5). Outside this layer are the swollen cells of the former vaginal wall. Layers of cells with a similar appearance have been found in the epidermis of the mouse by Hanson (1947), who points out that these various layers are not always distinct; for example, dividing cells may be found in the stratum spinosum for a short period after its formation, and before the cell cytoplasm has increased in quantity. Similar cells may be found in the vaginal epithelium.

Changes of a similar type are seen in the normal intact animal.

Periodic acid-Schiff technique (*PAS*)

It has been generally accepted that periodic acid oxidation forms aldehyde from 1,2-glycol linkages (—CHOH—CHOH—). However, —CHOH—$CHNH_2$— and —CHOH—CHNHR— groupings are also oxidized with the formation of aldehydes (Meyer, Odier & Siegrist, 1948; Jeanloz & Forchielli, 1951; Lillie, 1950, 1951*b*). In tissue sections these aldehydes can be demonstrated with Schiff's reagent, and the method is now widely used as a histochemical test for polysaccharides, whether they are free or in combination with proteins. Recently, McManus & Cason (1950) have introduced an acetylation technique in which treatment of sections with acetic anhydride in pyridine blocks the glycol groups and prevents the production of aldehydes; the acetylation can be reversed by subsequent treatment with potassium hydroxide. They claim that the prevention of the PAS reaction by acetylation provides additional proof that a carbohydrate is present in the sections.

In using this technique for the demonstration of carbohydrates two control procedures must be adopted. Free aldehydes may be found in the sections, and their presence can be detected by omitting the periodic acid oxidation step in the PAS method. Also, Hack (personal communication to Gersh, 1949) and Wolman (1950) have shown that many free unsaturated lipids and sphingolipids may recolorize Schiff's reagent after periodic acid oxidation. These free lipids can be extracted from sections with hot 1:1 methanol-chloroform (Gersh, 1949).

Four regions react with the PAS technique in the vaginal wall of both ovariectomized and normal animals (Pl. 1, figs. 6–9):

(1) the former vaginal epithelium beneath which keratinization occurs,
(2) the cytoplasm of the cells forming the stratum spinosum,
(3) the basement membrane,
(4) the intercellular ground substance.

Table 1 gives a summary of the results obtained after the treatment of sections with various reagents and enzymes.

Table 1

	Former epithelium	Stratum spinosum	Basement membrane	Inter-cellular ground substance
No periodate treatment	—	—	—	—
Acetic anhydride + pyridine	—	—	—	—
Acetic anhydride + pyridine followed by KOH	+	+	+	+
Methanol:chloroform (1:1), 16 hr.	+	+	+	+
Saliva (1:4), 1 hr.	+	—	+	+
Diastase (1% in acetate buffer pH5), 1 hr.	+	—	+	+
Pepsin (0·1 mg./ml. in 0·01N-HCl), 5 min.	+	+	+	+
Pepsin (5 mg./ml. in 0·01N-HCl), 1 hr.	+	+	—	—

+ indicates a positive PAS reaction.

(1) *Former vaginal wall*

The cells of this layer become very swollen and stain intensely with the PAS technique. The results shown in Table 1 demonstrate the carbohydrate nature of the mucinous material present in these cells. The fact that the strong pepsin solution does not destroy this material, while it does destroy the staining of the intercellular ground substance, indicates that the material is not linked to any appreciable amount of protein. A similar substance has been demonstrated in the acrosome of the sperm of the Hemipteran insect, *Arvelius albopunctatus*, by Schrader & Leuchtenberger (1951), who concluded that the substance was a mucopolysaccharide.

(2) *Stratum spinosum*

The results shown in Table 1 demonstrate that the PAS-positive material in this layer is glycogen. Table 2 shows the results from material obtained at various stages after the injection of oestrone.

Table 2. *The time of deposition of glycogen in the stratum spinosum*

Time after injection (hr.)	Intravaginal	Subcutaneous
36	—	—
48	Trace*	Trace*
60	Trace	Trace
72	+ +†	+ +†
84	+ +	+ +

* Commencement of keratinization. † Fully keratinized.

In intact animals glycogen deposition occurs at the end of oestrus. It is seen that in both artificially stimulated and normal animals glycogen is deposited in the cells of the stratum spinosum when full keratinization of the vagina is attained (Pl. 1, figs. 7, 9). The glycogen is in a granular state and in places forms caps on the poles of the nuclei. However, the observed form of the glycogen is probably an artefact, since recent freeze-drying techniques indicate that glycogen is distributed diffusely throughout the cytoplasm (Mancini, 1948; Lison & Vokaer, 1949).

(3) *Basement membrane*

At all stages a prominent basement membrane is demonstrated by the PAS technique, although Allen (1922) reported the absence of a basement membrane in the dioestrous and ovariectomized animal. No difference in its structure could be demonstrated during the cornification process. Its destruction by strong pepsin solution suggests it contains a glycoprotein.

(4) *Intercellular ground substance*

The PAS reaction was observed in all sections examined, although its intensity was not as pronounced as in the former vaginal wall or the glycogen of the stratum spinosum. Oestrogens seemed to have no effect upon the staining reaction. Its destruction by strong pepsin solution suggests that the ground substance contains a glycoprotein.

Metachromasia with toluidine blue

Many papers have been published on the metachromatic staining of various tissue components with basic dyes (the relevant literature has been reviewed by Dempsey & Wislocki, 1946; Dempsey, Bunting, Singer & Wislocki, 1947; Wislocki, Bunting & Dempsey, 1947; and Bunting, 1949). Aspects of the chemical basis of the reaction have been discussed by Michaelis & Granick (1945), Michaelis (1947) and Landsmeer (1951). At present it is impossible to list all substances which stain metachromatically. Lison (1936) was the first to recognize the main group; he states that they are substances which are esters of sulphuric acid with carbohydrate of a high molecular weight, sometimes combined with a protein and sometimes not, e.g. chondroitin sulphuric acid and mucoitin sulphuric acid. Lison's statement is not the whole truth, since phosphoric acid or carbonic acid can be substituted for sulphuric acid above (Bank & de Jong, 1939).

While all workers are unanimous in the belief that all sulphate-containing compounds are strongly metachromatic there is a controversy as to whether or not hyaluronic acid produces metachromasia. Several authors maintain that hyaluronic acid does not produce metachromasia (see Lillie, Emmart & Laskey, 1951, for a review); Dempsey & Wislocki and their co-workers, however, have maintained that it does. The main difficulty in this work is that no pure preparations of the various mucopolysaccharides are available; this is well illustrated in a recent paper (Rapport, Meyer & Linker, 1951) where the 'pure' hyaluronic acid preparation had about 12 % of sulphated polysaccharide impurity. The conclusion must be reached that the metachromatic test is not molecule specific. However, the test is useful in detecting regions of acid mucopolysaccharides and mucoproteins and assists in the interpretation of other histochemical tests.

In the present work a 0·5 % aqueous solution of toluidine blue has been used.

Only one region of the vaginal wall stains metachromatically with toluidine blue. This is the former vaginal wall which, as noted earlier, stains with the PAS technique. The metachromatic staining, however, is not alcohol-resistant. The intercellular ground substance fails to stain metachromatically, although metachromatic mast cells are distributed throughout. In these cells the staining reaction is alcohol-resistant.

DISCUSSION

Epithelial mucin

The PAS-positive nature of the vaginal mucin of rodents has been mentioned by Leblond (1950) and Lillie (1951 *b*). In the present work, two main histochemical properties of this material have been demonstrated. First, the material stains with the PAS technique and is not destroyed by strong solutions of pepsin, and secondly, the material is weakly metachromatic. The first property indicates that the substance is a mucopolysaccharide. Lillie (1951 *b*) has suggested that substances which react strongly with the PAS technique and which are weakly metachromatic may be relatively short-chained polysaccharides.

Preliminary studies on the mucinous substances found in the vagina of the mouse during pregnancy show that the material is the same as that described above. Further studies on the nature of these mucinous substances are in progress.

Keratinization occurs in the flattened cells which lie immediately below the mucified layer. Although this fact was described by the pioneer workers in the field, in recent times it seems to have passed unnoticed. The phenomenon demonstrates that oestrogens cause a unique and rapid differentiation of cells of the vaginal epithelium, the main feature of the differentiation being the ability to produce keratin. Early views on keratinization were to the effect that it is a drying up process caused by the removal of cells from their source of nutriment, full keratinization being attained upon death of the cell. This theory is quite untenable in the case of the vagina where the superficial cells do not keratinize. While undoubtedly the fully keratinized cells are dead the cause of death is not desiccation but the excessive synthesis of keratin, i.e. the cells die as the result of their own activity. This view of keratinization as an active anabolic process is consistent with the demonstration of increased amounts of ribonucleic acid in the keratinizing vagina (Jeener, 1948), skin (Leuchtenberger & Lund, 1951), and hair follicles (Hardy, 1952).

Glycogen

The presence of glycogen in the vaginal mucosa is well known. Robertson, Maddux & Allen (1930) showed in the monkey that the glycogen content increases as cornification proceeds. Van Dyke & Ch'en (1936) in biochemical studies on the macaque showed similar changes. They also showed that ovariectomy causes a large reduction in the glycogen content of the mucosa, and that it is deposited in large amounts following injections of oestrone. Bullough & Eisa (unpublished observations quoted by Bullough & Van Oordt, 1950) have also shown the deposition of glycogen in the vagina of the mouse during the oestrous cycle. The studies reported in the present paper have shown that glycogen is deposited after the attainment of full keratinization, which is subsequent to the peak of mitotic activity reported by Allen, Smith & Gardner (1937). This is of interest in view of the importance of carbohydrates in the mechanisms involved in mitosis (Bullough, 1952). Glycogen is also a prominent constituent of growing stratified epithelium in other parts of the body, e.g. embryonic skin (Bernard, 1859), healing wounds (Bradfield, 1951) and skin grafts (Scothorne & Scothorne, 1953). In addition to its possible role in mitosis, glycogen may also play an important function in the keratin synthesis which occurs in all the above tissues. Bradfield (1951) has recently discussed this latter problem.

Intercellular ground substance

Two main histochemical properties characterize this material. First the substance is PAS-positive and can be destroyed by strong pepsin solution, and secondly, it fails to stain metachromatically with toluidine blue. Recently, Lillie *et al.* (1951) have suggested that regions which are PAS-positive and fail to stain metachromatically contain predominantly hyaluronic acid. A further example of this is provided by the vitreous body. Such an interpretation, however, is not consistent with the recent observation of Davies (1952), who has shown that a sulphate-free preparation of hyaluronic acid does not give a PAS-positive reaction following its deposition on microscopic slides. At the moment, therefore, the staining reactions cannot be attributed to any specific substance.

Two other regions of connective tissue lying beneath stratified squamous epithelium, and highly sensitive to oestrogenic stimulation, have been studied by histochemical techniques.

(1) *Sex skin of the monkey*

Duran-Reynals, Bunting & Van Wagenen (1950), following the earlier work of Chain & Duthie (1940), have studied the changes which occur in the ground substance of the sex skin of *Macaca mulatta*; by means of testicular and streptococcal hyaluronidases they claim to have demonstrated changes in the hyaluronic acid content of the skin. Their arguments depend on (i) changes in the metachromatic staining reaction, and (ii) the destruction of most of the metachromasia by streptococcal hyaluronidase; the bacterial enzyme is believed to be exclusively a hyaluronidase, while the testicular one is believed to contain in addition a chondroitinase. This work cannot be regarded as conclusive, other than that it demonstrates changes in the degree of metachromasia. There is no general agreement that hyaluronic acid can cause metachromasia, and recent reports indicate that streptococcal hyaluronidase does not consist exclusively of that enzyme (Lillie *et al.* 1951; Meyer & Rapport, 1952).

(2) *Vagina of the rat*

Recently, Bostrom & Odeblad (1952) have shown by autoradiographic means that in the rat vagina there is an accumulation of ^{32}S following oestrogenic stimulation. They believe that this represents an increased formation of chondroitin sulphate. As yet neither metachromasia nor the PAS reaction have been specifically studied in the rat vagina. If chondroitin sulphate is formed, metachromasia of the intercellular ground substance should be observed, in contrast to the observations reported in the mouse.

The above results show that connective tissues which are highly sensitive to oestrogens have different histochemical properties. Until all the various techniques have been applied to at least one species of animal interpretation of results at the chemical level is impossible. Further studies on this problem are in progress.

SUMMARY

1. Four regions of the vagina of the mouse react positively to the periodic acid oxidation Schiff procedure: (i) the former vagina wall, (ii) the cells of the stratum spinosum, (iii) the basement membrane, and (iv) the intercellular ground substance. The only region of metachromasia is the former vaginal wall. The metachromasia is not alcohol-resistant.

2. The mucinous material of the former vaginal epithelium is not lipoidal in nature, and is resistant to salivary amylase, diastase and strong pepsin solutions. The material only forms during cornification and beneath it keratinization occurs. Following completion of cornification the layer is sloughed off. Non-keratinization of these superficial cells is strong evidence that keratinization is an active anabolic process.

3. The PAS-positive material in the stratum spinosum is glycogen and is deposited at the end of cornification.

4. No differences have been shown between the carbohydrate components of the vagina during cornification in animals undergoing normal cycles and in ovariectomized animals stimulated by either the subcutaneous or intravaginal administration of oestrone.

I wish to express my thanks to Prof. C. W. Emmens for the interest he has taken in this work, which was aided by grants from the Commonwealth Bank of Australia and the Wool Research Trust Fund. Also, I am indebted to Mr C. R. Austin for help in the preparation of the plate.

REFERENCES

Allen, E. (1922). The oestrous cycle in the mouse. *Amer. J. Anat.* **30**, 297–371.

Allen, E. & Doisy, E. A. (1923). An ovarian hormone: preliminary report on its localization, extraction and partial purification and action in test animals. *J. Amer. med. Ass.* **81**, 819–821.

Allen, E., Francis, B. F., Robertson, L. L., Colgate, C. E., Johnston, C. G., Doisy, E. A., Kountz, W. B. & Gibson, H. V. (1924). The hormone of the ovarian follicle; its localization and action in test animals, and additional points bearing upon the internal secretion of the ovary. *Amer. J. Anat.* **34**, 133–181.

Allen, E., Smith, G. M. & Gardner, W. U. (1937). Accentuation of the growth effect of theelin on genital tissues of the ovariectomized mouse by arrest of mitosis with colchicine. *Amer. J. Anat.* **61**, 321–341.

Bank, O. & Bungenberg de Jong, H. G. (1939). Untersuchungen über Metachromasie. *Protoplasma*, **32**, 489–516.

Bernard, C. (1859). De la matière glycogène considérée comme condition de développement de certains tissues, chez le foetus, avant l'apparition de la fonction glycogénique du foie. *C.R. Acad. Sci., Paris*, **48**, 673–684.

Biggers, J. D. (1951). Observations on the intravaginal assay of natural oestrogens using aqueous egg albumin as the vehicle of administration. *J. Endocrin.* **7**, 163–171.

Biggers, J. D. (1952). The response of the vaginal epithelium of the ovariectomized mouse to the systemic and local administration of oestrone. *Nature, Lond.*, **170**, 895–896.

Bostrom, H. & Odeblad, E. (1952). Autoradiographic observations on the uptake of ^{35}S in the genital organs of the female rat and rabbit after injection of labelled sodium sulphate. *Acta endocr., Copenhagen*, **10**, 89–96.

Bradfield, J. R. G. (1951). Glycogen of vertebrate epidermis. *Nature, Lond.*, **167**, 40–41.

Bullough, W. S. (1952). The energy relations of mitotic activity. *Biol. Rev.* **27**, 133–168.

Bullough, W. S. & Van Oordt, G. J. (1950). The mitogenic actions of testosterone propionate and of oestrone on the epidermis of the adult male mouse. *Acta endocr., Copenhagen*, **4**, 291–305.

Bunting, H. (1949). The distribution of acid mucopolysaccharides in mammalian tissue as revealed by histochemical methods. *Ann. N.Y. Acad. Sci.* **52**, 977–982.

Chain, E. & Duthie, E. S. (1940). Identity of hyaluronidase and spreading factor. *Brit. J. exp. Path.* **21**, 324–338.

Davies, D. V. (1952). Specificity of staining methods for mucopolysaccharides of the hyaluronic acid type. *Stain Tech.* **27**, 65–70.

Dempsey, E. W., Bunting, H., Singer, M. & Wislocki, G. B. (1947). The dye-binding capacity and other chemo-histological properties of mammalian mucopolysaccharides. *Anat. Rec.* **98**, 417–429.

Dempsey, E. W. & Wislocki, G. B. (1946). Histochemical contributions to physiology. *Physiol. Rev.* **26**, 1–27.

Duran-Reynals, F., Bunting, H. & van Wagenen, G. (1950). Studies on the sex skin of *Macaca mulatta*. *Ann. N.Y. Acad. Sci.* **52**, 1006–1014.

Emmens, C. W. (1950). *Hormone Assay*, pp. 405–406. New York: Academic Press.

Foraker, A. G. & Brawner, D. L. (1951). Quantitative exfoliative cytology: differential counting of cervical smears stained for glycogen in cases of pregnant and non-pregnant women. *Arch. Path.* (*Lab. Med.*) **51**, 201–204.

Freud, J. (1939). Diethylstilboestrol and other oestrogenic compounds compared. Intravaginal route. *Acta brev. neerl. Physiol.* **9**, 11–13.

Gersh, I. (1949). A protein component of the Golgi apparatus. *Arch. Path.* (*Lab. Med.*) **47**, 99–109.

Hanson, J. (1947). The histogenesis of the epidermis in the rat and mouse. *J. Anat., Lond.*, **81**, 174–197.

Hardy, M. H. (1952). The histochemistry of hair follicles in the mouse. *Amer. J. Anat.* **90**, 285–338.

Jeanloz, R. W. & Forchielli, E. (1951). Studies on hyaluronic acid and related substances. II. Periodate oxidation of glucosamine and derivatives. *J. biol. Chem.* **188**, 361–369.

Jeener, R. (1948). Acides nucléiques et phosphatases au cours de phénomènes de corissance provoqués par l'oestradiol et la prolactine. *Biochim. biophys. Acta*, **2**, 439–453.

Kamell, S. A. & Atkinson, W. B. (1948). Effects of ovarian hormones on certain cytoplasmic reactions in the vaginal epithelium of the mouse. *Proc. Soc. exp. Biol., N.Y.*, **68**, 537–540.

Landsmeer, J. N. F. (1951). Some colloid chemical aspects of metachromasia. Influence of pH and salts on metachromatic phenomena evoked by toluidine blue in animal tissue. *Acta physiol. pharm. neerl.* **2**, 112–128.

Leblond, C. P. (1950). Distribution of periodic acid-reactive carbohydrates in the adult rat. *Amer. J. Anat.* **86**, 1–25.

Leuchtenberger, C. & Lund, H. Z. (1951). The chemical nature of the so-called keratohyaline granules of the stratum granulosum of the skin. *Exp. Cell Res.* **2**, 150–152.

Lillie, R. D. (1950). Further exploration of the HIO_4-Schiff reaction with remarks on its significance. *Anat. Rec.* **108**, 239–253.

Lillie, R. D. (1951*a*). The allochromy procedure. *Amer. J. Clin. Path.* **21**, 484–488.

Lillie, R. D. (1951*b*). Histochemical comparison of the Casella, Bauer and periodic acid oxidation-Schiff leucofuchsin technics. *Stain Tech.* **26**, 123–136.

Lillie, R. D., Emmart, E. W. & Laskey, A. M. (1951). Chondromucinase from bovine testis and the chondromucin of the umbilical cord. *Arch. Path.* (*Lab. Med.*), **52**, 363–368.

Lison, L. (1936). *Histochimie Animale*, pp. 236–242. Paris: Granthier-Villars.

Lison, L. & Vokaer, R. (1949). Sur la détection histochimique du glycogène des cellules vaginales chez la femme. *Ann. Endocr., Paris*, **10**, 66–72.

Loewe, S. & Voss, E. H. V. (1926). Eine placentare Inkretdrüse spenderin örtlich wirksamen Hormons? *Klin. Wschr.* **5**, 1083–1085.

Long, J. A. & Evans, H. M. (1922). The oestrous cycle in the rat and its associated phenomena. *Mem. Univ. Calif.* **6**, 1–148.

McManus, J. F. A. (1946). Histological demonstration of mucin after periodic acid. *Nature, Lond.*, **158**, 202.

McManus, J. F. A. & Cason, J. E. (1950). Carbohydrate histochemistry studied by acetylation techniques. I. Periodic acid methods. *J. exp. Med.* **91**, 651–654.

Mancini, R. E. (1948). Histochemical study of glycogen in tissues. *Anat. Rec.* **101**, 149–159.

Meyer, K. H., Odier, M. E. & Siegrist, A. E. (1948). Constitution de l'acide chondroïtine-sulfurique. *Helv. chim. acta*, **31**, 1400–1419.

Meyer, K. & Rapport, M. M. (1952). Hyaluronidases. *Advanc. Enzymol.* **13**, 199–236.

Michaelis, L. (1947). The nature of the interaction of nucleic acids and nuclei with basic dyestuffs. *Cold Spr. Harb. Symp. quant. Biol.* **12**, 131–142.

Michaelis, L. & Granick, S. (1945). Metachromacy of basic dyestuffs. *J. Amer. chem. Soc.* **67**, 1212–1219.

Morau, H. (1889). Des transformations epitheliales de la muqueuse du vagin de quelques ronguers. *J. Anat., Paris*, **25**, 275–297.

Rapport, M. M., Meyer, K. & Linker, A. (1951). Analysis of the products formed on hydrolysis of hyaluronic acid by testicular hyaluronidase. *J. Amer. chem. Soc.* **73**, 2416–2420.

Retterer, E. (1892). Evolution de l'epithelium du vagin. *C.R. Soc. Biol., Paris*, **44**, 566–568.

Robertson, D. C., Maddux, W. P. & Allen, E. (1930). Ovarian hormone effects in ovariectomized monkeys. *Endocrinology*, **14**, 77–88.

Schrader, F. & Leuchtenberger, C. (1951). The cytology and chemical nature of some constituents of the developing sperm. *Chromosoma*, **4**, 404–428.

Scothorne, R. J. & Scothorne, A. W. (1953). Histochemical studies on human skin autografts. *J. Anat., Lond.*, **87**, 11–29.

Stockard, C. R. & Papanicolaou, G. N. (1917). The existence of a typical oestrous cycle in the guinea-pig—with a study of its histological and physiological changes. *Amer. J. Anat.* **22**, 225.

Van Dyke, H. B. & Ch'en, G. (1936). Observations on the biochemistry of the genital tract of the female macaque particularly during the menstrual cycle. *Amer. J. Anat.* **58**, 473–499.

Wislocki, G. B., Bunting, H. & Dempsey, E. W. (1947). Metachromasia in mammalian tissues and its relationship to mucopolysaccharides. *Amer. J. Anat.* **81**, 1–38.

Wolman, M. (1950). Staining of lipids by the periodic-acid-Schiff reaction. *Proc. Soc. exp. Biol., N.Y.*, **75**, 583–585.

EXPLANATION OF PLATE

Fig. 1. Vaginal wall of an untreated ovariectomized mouse. Haematoxylin and eosin. × 280.

Fig. 2. Vaginal wall of an ovariectomized mouse 24 hr. after the subcutaneous injection of 0·5 μg. oestrone. Haematoxylin and eosin. × 280. The inner large 'mucified' cells represent the former vaginal wall; the cells beneath are newly formed cells.

Fig. 3. Vaginal wall of an ovariectomized mouse 24 hr. after the intravaginal injection of 0·002 μg. oestrone. Haematoxylin and eosin. × 280. The cellular pattern of the epithelium is similar to that of fig. 2.

Fig. 4. Vaginal wall of an ovariectomized mouse 48 hr. after the subcutaneous injection of 0·5 μg. oestrone. Haematoxylin and eosin. × 280. Keratinization can be seen in the layer of cells immediately below the superficial mucified cells.

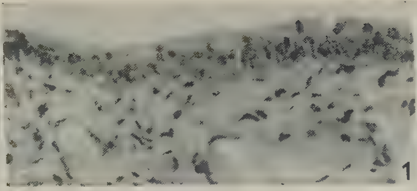
Fig. 5. Vaginal wall of an ovariectomized mouse 48 hr. after the intravaginal injection of 0·002 μg. oestrone. Haematoxylin and eosin. × 280. The cellular pattern of the epithelium is similar to that of fig. 4, although at a somewhat earlier stage.

Fig. 6. Vaginal wall of an ovariectomized mouse 36 hr. after the intravaginal injection of 0·002 μg. oestrone. PAS technique. × 665. The mucified cells of the former vaginal wall and the basement membrane have reacted strongly (dark regions), while the intercellular ground substance has reacted to a lesser degree. No PAS-positive material is seen in the stratum spinosum.

Fig. 7. Vaginal wall of an ovariectomized mouse 72 hr. after the intravaginal injection of 0·002 μg. oestrone. PAS technique. × 665. The positive reaction is seen in the basement membrane and intercellular ground substance. In addition, PAS-positive material is seen in the stratum spinosum. Enzymatic tests have shown this material to be glycogen.

Fig. 8. Vaginal wall of an ovariectomized mouse 48 hr. after the subcutaneous injection of 0·5 μg. oestrone. PAS technique. × 665. The mucified cells, the basement membrane and intercellular ground substance has reacted strongly. Traces of PAS-positive material (glycogen) are seen in the stratum spinosum.

Fig. 9. Vaginal wall of an ovariectomized mouse 84 hr. after the subcutaneous injection of 0·5 μg. oestrone. PAS technique. × 665. Positive reactions are seen in the basement membrane and intercellular ground substance. Also a large deposition of glycogen is seen in the stratum spinosum.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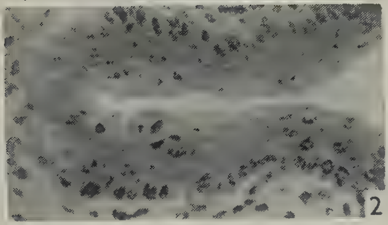
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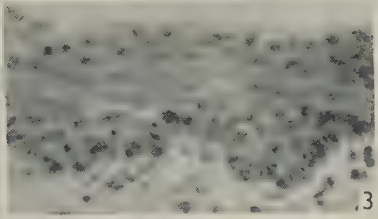
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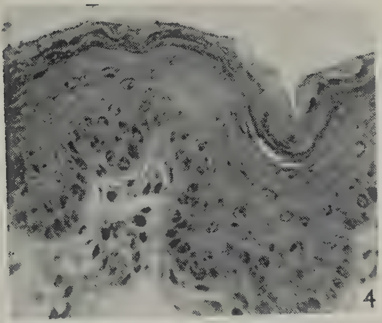
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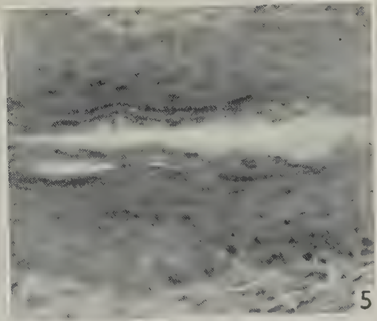
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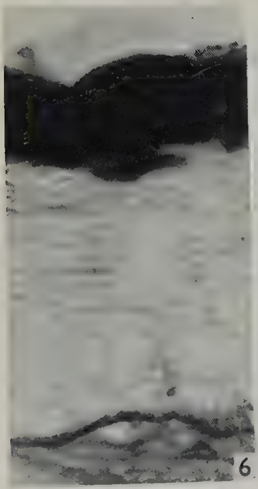
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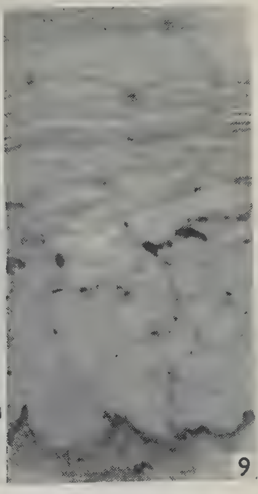
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BIGGERS—Carbohydrate components of the vagina of the mouse

THE MUSCLE-CARTILAGE RELATIONSHIP IN THE EXTRAPULMONARY BRONCHI

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The mammalian trachealis muscle lies in the posterior membranous portion of the trachea, where its interlacing bundles of smooth muscle fibres connect together the free extremities of the cartilaginous crescents. The muscle fibres are attached directly to the perichondrium (Miller, 1913), the attachment being in some species (e.g. cat, dog, rabbit) to the external aspect of the crescents, in some (e.g. guinea-pig, hedgehog, man) to their internal aspect, and in others (e.g. rat) to the tip and both aspects of the crescents. This species variation has been reported by many investigators (Cuvier, 1805; Meckel, 1833; Luschka, 1869; Verson, 1871; Stirling, 1883; Guieysse, 1898; Miller, 1913), but its significance is unknown.

On the other hand, the muscle of Reisseisen of the intrapulmonary bronchi is arranged in the form of a branched tubular net, a 'geodesic network' (Miller, 1921), which, completely surrounding the lumen, is situated internally to the bronchial cartilages without obtaining any direct attachment to them. It is said to have the same structural arrangement in all mammals, except in Cetaceans, where the specialized form of the muscle is possibly connected with the mechanism of submersion (Baudrimont, 1929).

The interesting problem of where in the tracheo-bronchial tree and in what manner the change-over occurs from the one muscular arrangement to the other appears to have received but little attention, and in authoritative works on the anatomy of this region either the subject is not dealt with at all or else the descriptions given are vague and incomplete. Current text-books of anatomy and histology (e.g. Bouin, 1932; Bremer & Weatherford, 1948; Bucher, 1948; Schafer, 1949; Gray, 1950; Ham, 1950; Cunningham, 1951; Maximow & Bloom, 1952) do not refer to the matter and usually describe the main bronchi as being identical in structure with the trachea. In the monographs of Engel (1947), Miller (1950) and Bariéty, Paillas & Lévy (1951) no mention is made of the way in which the change-over is effected. Even in von Möllendorff's *Handbuch*, where Heiss (1936) deals with the respiratory tract, only brief reference is made to the question; this author says that in man the structure of the bronchial wall changes at the level of the origin of the upper lobe bronchus on the left and just above the bronchus to the middle lobe on the right, and that from these levels on a rearrangement (Umschichtung) takes place, such that cartilages with connecting fibrous tissue come to surround the whole circumference of the tube and the musculature to form its 'own layer' internal to and independent of the cartilages. Macklin (1929), too, speaks of a gradual modification of structure occurring 'as we pass to the stem bronchi', but his description is vague and in part inaccurate.

It seems that the only investigator who has made a special study of the problem is Guieysse (1896, 1898), and, since the results of the present work are in certain respects at variance with his findings, it is necessary to go into some detail concerning his account. He studied serial sections of rabbit and guinea-pig bronchi, and single sections at various levels of the respiratory tract in other common mammals. On the basis of his findings, which he says are applicable to all species examined, he maintains that the extrapulmonary bronchi are identical in structure with the trachea, and that modifications begin only 'at the hilum' of the lung; indeed, the bronchus to the lower lobe is described by him as entering the lung and traversing it for some distance whilst still having the structural characteristics of an extrapulmonary bronchus. But his main contention is that the pattern of modification of the muscular arrangement is completely different in the lower lobe bronchus compared with that found in the bronchi to the upper and middle lobes; in the former (lower lobe bronchus) the muscle bundles, arranged at first as in the trachea, gradually extend around the lumen and are slowly transformed into a muscle of Reisseisen, whereas in the latter (upper and middle lobe bronchi) the muscle of Reisseisen appears quite suddenly, being from the outset completely independent of the muscle of the 'stem' bronchus. The first portions of these bronchial branches are, according to Guieysse, entirely devoid of muscle. Moreover, he states specifically that in the cat and the dog the arrangement is as described for other species, but he seems to have had difficulty in tracing the modification in these animals, since he says that 'le passage se fait sur un très petit espace et il est bien difficile de tomber sur ce point'. Apart from Oppel (1905), who quotes the work of Guieysse in some detail, later writers appear to have overlooked his contribution to the subject.

Study of the literature thus reveals a rather curious gap in the descriptions of the microscopic anatomy of this region, and since the techniques employed by Guieysse and his method of approach to the subject appeared to be not altogether satisfactory, the matter has been reinvestigated. The best way to obtain a clear picture of structural modifications which occur so rapidly and in so complex a manner was clearly to construct, from serial sections, a model of the bronchi to show the relationship between cartilage and muscle. Once this had been completed and the significance of various microscopic features established, it then became possible to identify similar features in sections from other specimens and to interpret them with confidence; species variations are such, however, that one hesitates to generalize about some of the findings without first constructing models of this region from many forms.

MATERIALS AND METHODS

The present study was performed mainly on the bronchi of two 4-day-old cats. In this species the trachealis muscle is attached to the external aspect of the tracheal crescents, a feature which facilitates differentiation both between the two types of muscular arrangement and between the two 'types' of cartilage.* Young animals were studied in order that the whole area of tissue could be conveniently handled for reconstruction purposes. The animals were killed with chloroform, and the trachea and lungs dissected free and fixed in Helly's fluid. After trimming, the specimens were embedded in paraffin, being orientated so that in the one case the bronchus to the right lung was sectioned transversely and in the other the bronchus to the left lung. Serial sections at 10μ were stained with Masson's trichrome stain and mounted in Canada balsam. From the series of sections of the bronchus to the left lung a reconstruction was made at a magnification of × 62 and using a technique modified from Gage (1907), to show the relative dispositions of cartilage and muscle throughout the region of the change-over. The model was built in separate portions and so could easily be dismantled for study (Pl. 1, figs. 1, 2). Its theoretical length was 136·4 mm., representing 2·2 mm. of tissue; its actual length when assembled was 142 mm. No further reference will be made in this paper to distances measured along or across a bronchus, since in a structure capable of considerable variation in both length and diameter it is not possible to assess the degree of distortion occasioned by removal from the thorax in the fresh state and by subsequent shrinkage due to fixation.

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* In this account the following modification of the terminology of Guieysse is used: where cartilage is arranged as in the trachea (i.e. in crescentic form with muscle attached directly to its external aspect) it is referred to as 'tracheal type' of bronchial cartilage, whilst the term 'bronchial cartilage' will be used only with reference to cartilages placed outside the muscle in the position typical for intrapulmonary bronchi.

Serial sections have also been studied of the bronchi of the guinea-pig and rabbit, and isolated sections of the bronchi of the dog and adult cat.

OBSERVATIONS

1. The model of the bronchi of the cat will be described first:

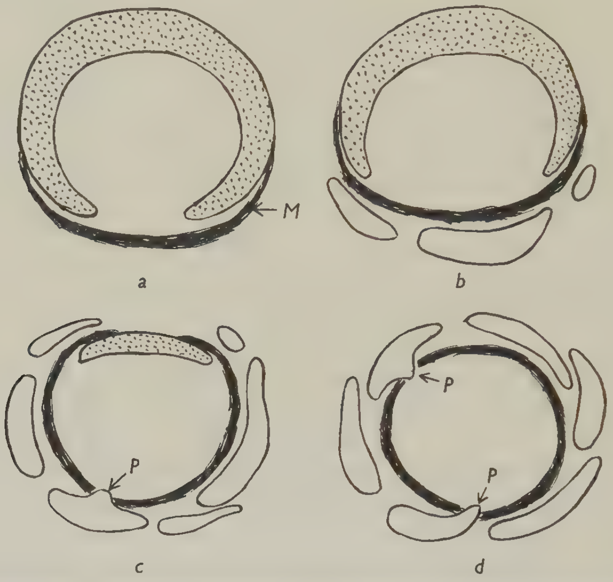
(1) The transition from the one muscular pattern to the other occurs entirely in the extrapulmonary portion of the bronchial tree, if one defines as an extrapulmonary bronchus one in which the peribronchial connective tissue is not directly surrounded by lung tissue but shows pleura intervening at some point. Indeed, Guieysse's statement must be reversed: the bronchi are for some distance extrapulmonary with the characteristics of intrapulmonary bronchi. No difficulty was found, using serial sections of extrapulmonary bronchi, in 'chancing upon' the region of transition, such as Guieysse noted when he used isolated sections from the cat and dog.

(2) The way in which the change-over is effected is identical for both upper and lower lobe bronchi, there being at the point of bifurcation of the main bronchus a division of all layers of the bronchial wall. Cartilages and muscle-bundles then continue their process of structural rearrangement in identical fashion for each bronchus (Pl. 1, figs. 2, 3).

(3) The pattern of formation of the muscle of Reisseisen is as follows: in the proximal portion of the main bronchus the arrangement of cartilage and muscle is identical with that in the trachea (Text-fig. 1*a*), but just above the level of origin of the bronchus to the upper lobe the bundles of muscle fibres corresponding to the trachealis muscle start gradually to extend farther round the lumen of the tube in proportion as their 'cartilages of insertion' (i.e. crescents of the tracheal type) diminish in size. At the same time bronchial cartilages appear outside the layer of muscle, at first posteriorly (Text-fig. 1*b*) and then over an increasing area as the muscle extends around the tube (Text-fig. 1*c*). Finally, the last vestige of a 'tracheal type' of bronchial cartilage disappears, the muscle bundles encircle the bronchus and all the cartilages are disposed outside the muscle as in an intrapulmonary

bronchus (Text-fig. 1 *d*). The structural modification is identical in both bronchi, but the transition is more rapid in the bronchus to the upper lobe.

(4) Bronchial cartilages of the tracheal type are often fused at some point, and the 'true' bronchial cartilages also branch and sometimes anastomose with one another; but nowhere is there to be seen a direct connexion between these two cartilage 'systems'. It is inaccurate therefore to speak of the whole series of bronchial cartilages as having at first the form of crescents which then 'break up' into irregular plaques.



Text-fig. 1. Diagrams of a series of transverse sections through an extrapulmonary bronchus of the kitten to illustrate the muscle-cartilage relationship at successive levels. Cartilages of the tracheal type are stippled. *M* represents the plane of the interlacing bundles of smooth muscle fibres; *P*, projections of bronchial cartilages to which the muscle is anchored.

(5) In the region of formation of a definitive muscle of Reisseisen, and apparently only in this region, there occur internal projections of the bronchial cartilages to which the bundles of muscle fibres appear to be closely anchored (Text-fig. 1 *c*, *d*; Pl. 1, figs. 2, 3; Pl. 2, figs. 6, 7, 8). These projections can be found at various points around the circumference of the tube, and have the form either of simple tuberosities, of projecting hooks or of half-wheels connecting one cartilage to the next one lower down. The bundles of muscle fibres appear to be anchored or 'hooked' by these projections, a phenomenon not seen in an intrapulmonary bronchus. Usually the projection is situated about the middle of the body of a cartilaginous plaque, but sometimes (as in Pl. 2, fig. 8) it is formed by the end of a plaque curving inwards. If the projection has the form of a hook it will then appear, in certain sections, to be quite separate from the cartilage with which it is connected (Pl. 2, fig. 7).

2. Some of these observations were confirmed by the study of other material. In the guinea-pig, and in the bronchus to the right lung of the kitten, the change-over is

already completed before the bronchus enters the lung substance. Extrapulmonary bronchi of the dog and adult cat sectioned near their entry into the lung have a fully formed muscle of Reisseisen (Pl. 2, fig. 9). In the rabbit the changes in muscle-cartilage relationship extend over a greater distance than in other species examined, and the bronchus enters the lung before the change-over is completely effected.

The second observation, that the pattern of modification is identical in different bronchi, could be confirmed for the right upper, middle and lower lobe bronchi of the kitten, except that the transition was already almost completed at the level of origin of the bronchus to the middle lobe. The same is true for the rabbit and probably also for the guinea-pig; in this animal, however, the cartilages are extremely thin, and extend for considerable distances around the lumen, with a great deal of overlapping and anastomosing. These features, together with the fact that the trachealis muscle itself lies internal to the tracheal type of cartilages near their tips, make analysis even of serial sections extremely difficult in this species.

The description of the way in which the muscle of Reisseisen is formed holds true for the rabbit and for the bronchus to the right lung of the kitten; also isolated sections of bronchi from the adult cat can best be interpreted on the same basis (Pl. 1, fig. 4).

With regard to the cartilaginous projections of the bronchial cartilages it was easy, after study of their various forms as seen on the model, to find similar features in the series of sections of the bronchus for the right lung of the kitten. In the case of the guinea-pig, because of the difficulties mentioned above one cannot be sure, without a reconstruction, of the true nature of what appear in some places to be attachments of muscle fibres to bronchial cartilages. In the rabbit the bundles of muscle fibres are certainly attached, in the region of transition, to the internal aspect of bronchial cartilages, but no typical projections as described for the kitten have been identified in this species.

DISCUSSION

The observations given in this paper of the way in which the trachealis muscle is modified to form the muscle of Reisseisen confirm the description given by Guieysse for the bronchus to the lower lobe. But the account given by this worker of structural differences between the lower lobe bronchus on the one hand and the bronchi to the upper and middle lobes on the other has not been confirmed, and on this point of disagreement there hang wider implications of a more theoretical nature.

The research of Guieysse was inspired, on his own admission, by the theory of monopodial branching of the bronchial tree advanced by Aeby (1880) and supported by D'Hardiviller (1897) in their respective fields of comparative anatomy and embryology, a theory which was gaining in popularity towards the end of the nineteenth century, and one with which Guieysse appears to have been much impressed. His preoccupation with this theory, and his apparent desire to find morphological evidence in favour of it, appear to have misled him in both his observations and conclusions. He argued, in support of the theory of monopodial branching, that his findings seemed to prove that the bronchus of the lower lobe, being markedly different in its structural arrangement from the bronchi to the upper and middle lobes, was truly 'la bronche souche de l'arbre respiratoire'. As a pictorial illustration of his findings he gives a drawing (Guieysse, 1898, fig. 3) of a transverse section of a 'stem' bronchus at the level of origin from it of the bronchus to the upper lobe. In this drawing the muscle of the upper lobe bronchus certainly appears to be completely independent of the muscle of the 'stem' bronchus, having from the outset all the characteristics of a muscle of Reisseisen, and leaving the first part of the tube entirely devoid of muscle. The correct interpretation of this picture would appear to be that the longitudinal section of the upper lobe bronchus has failed to include in its first portion any part of that comparatively small region in its circumference where muscle fibres are to be found; in the same way one could prepare a longitudinal section of a trachea apparently devoid of muscle fibres by cutting it in the coronal plane. Pl. 2, fig. 5, is a photograph of a section from a similar situation, cut in such a plane that the continuity of the muscle between the stem bronchus and the upper lobe bronchus is shown.

The present findings, then, do not give support to the theory of monopodial branching of the bronchial tree; if one were to argue any theory of branching from them one could only say that so far as the cartilage and muscle of the main bronchi are concerned the method appears to be one of 'uneven dichotomy'.

Functional interpretations of gross morphological findings in the respiratory tract can usually be no more than tentative, since, so far as its structure is concerned, the region is so difficult of access both for direct observation in the living animal and for application of the experimental method. However, if one takes the view that the whole tracheo-bronchial tree is 'dynamic' in nature, being subject to increase in both length and width during inspiration and to the reverse during expiration (Macklin, 1932), it seems reasonable that the musculature of this tree should be in the form of a continuous network, as here described, relatively fixed (by direct attachment to cartilage) in the region of the relatively immobile trachea, and becoming more 'labile' in those more distal parts of the system which are subject to the greatest changes in form and position. If this concept is true it is perhaps not unlikely that the projections, which appear to anchor the muscle to the extrapulmonary bronchial cartilages, are true 'muscular processes', and represent an intermediate stage in the degree of fixation of the muscular network.

SUMMARY

1. The change-over from the trachealis muscle to the muscle of Reisseisen has been investigated by the construction, from serial sections, of a model of the bronchus to the left lung of the kitten.

2. The rearrangement of muscle and cartilage in the region of transition is described in detail. It is found to be identical for both upper and lower lobe bronchi and to take place in their extrapulmonary portions.

3. Two topographically distinct series of bronchial cartilages are described.

4. The muscle of Reisseisen, when first formed, is anchored to the bronchial cartilages by a series of cartilaginous projections of varying shape.

5. Some of the findings are confirmed on sections from dog, adult cat, guinea-pig and rabbit.

6. Theoretical and functional implications of the findings are briefly discussed.

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REFERENCES

Aeby, Chr. (1880). *Der Bronchialbaum der Säugethiere und des Menschen nebst Bemerkungen über den Bronchialbaum der Vögel und Reptilien.* Leipzig: Wilhelm Engelmann.

Bariéty, M., Paillas, J. & Lévy, M. (1951). *La Trachée et les Bronches Cartilagineuses.* Paris: Masson et Cie.

Baudrimont, A. (1929). Dispositifs musculaires et élastiques du poumon des vertébrés. *Bull. Soc. sci. Arcachon.* **26**, 1–232.

Bouin, P. (1932). *Éléments d'Histologie*, vol. **2**. Paris: Libraire Félix Alcan.

Bremer, J. L. & Weatherford, H. L. (1948). *A Textbook of Histology*, 6th ed. Philadelphia: The Blakiston Co.

Bucher, O. (1948). *Histologie.* Bern: H. Huber.

Cunningham, D. J. (1951). *Textbook of Anatomy*, 9th ed. Edited by J. C. Brash. London: Oxford University Press.

Cuvier, G. (1805). *Leçons d'Anatomie Comparée*, **4**, 307–316. Paris: Raudouin.

D'Hardiviller. (1897). La ramification bronchique chez le lapin. *Bibliogr. anat.* **5**, 17–31.

Engel, S. (1947). *The Child's Lung.* London: E. Arnold and Co.

Gage, Susanna P. (1907). The method of making models from sheets of blotting paper. *Anat. Rec.* **1**, 166–169.

Gray, H. (1950). *Anatomy*, 30th ed. Edited by T. B. Johnston & J. Whillis. London: Longmans, Green and Co.

Guieysse, M. A. (1896). Muscle trachéal et muscles de Reisseisen. *C.R. Soc. Biol., Paris*, **10**, 897–899.

Guieysse, M. A. (1898). Sur quelques points d'anatomie des muscles de l'appareil respiratoire. *J. Anat., Paris*, **34**, 419–432.

Ham, A. W. (1950). *Histology.* Philadelphia: J. B. Lippincott Co.

Heiss, R. (1936). Der Atmungsapparat. In Möllendorff's *Handbuch der mikroskopischen Anatomie des Menschen*, Bd. **5**, Tl. 3, 747. Berlin: J. Springer.

Luschka, H. (1869). Die Muskulatur der Luftröhre des Menschen. *Arch. Anat. Physiol., Lpz.*, **14**, 589–596.

Macklin, C. C. (1929). The musculature of the bronchi and lungs. *Physiol. Rev.* **9**, 1–60.

Macklin, C. C. (1932). The dynamic bronchial tree. *Amer. Rev. Tuberc.* **25**, 393–417.

Maximow, A. A. & Bloom, W. (1952). *A Textbook of Histology*, 6th ed. Philadelphia: W. B. Saunders Co.

Meckel, J. F. (1833). *System der vergleichenden Anatomie*, 6th part, vol. entitled 'Athmungs- und Stimmwerkzeuge'. Halle: Buchhandlung des Waisenhauses.

Miller, W. S. (1913). The trachealis muscle. Its arrangement at the carina tracheae and its probable influence on the lodgement of foreign bodies in the right bronchus and lung. *Anat. Rec.* **7**, 373–385.

Miller, W. S. (1921). The musculature of the finer divisions of the bronchial tree and its relation to certain pathological conditions. *Amer. Rev. Tuberc.* **5**, 689–704.

Miller, W. S. (1950). *The Lung*, 2nd ed. Springfield, Illinois: C. C. Thomas.

Oppel, A. (1905). *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere*, Bd. **6**, Atmungsapparat, pp. 598–600. Jena: Fischer.

Schafer, E. A. (1949). *Essentials of Histology*, 15th ed. Edited by H. M. Carleton & E. H. Leach. London: Longmans, Green and Co.

Stirling, W. (1883). The trachealis muscle of man and animals. *J. Anat. Physiol.* **17**, 204–206.

Verson, E. (1871). Kehlkopf und Trachea. In Stricker's *Handbuch der Lehre von den Geweben des Menschen und der Tiere*, pp. 453–463. Leipzig: Wilhelm Engelmann.

EXPLANATION OF PLATES

Abbreviations: T = tracheal type of bronchial cartilage; Br = 'true' bronchial cartilage; M = muscle; P = projections of bronchial cartilage to which bundles of muscle fibres are anchored.

Plate 1

Fig. 1. Posterior view of reconstruction model of bronchi to left upper and lower lobes of the kitten, showing the muscle-cartilage relationship. In the photograph cartilage appears white and muscle black.

Fig. 2. Section through the model at the level of the line AB (fig. 1), viewed from the infero-lateral aspect. The bronchus to the upper lobe is seen above, and that to the lower lobe, with a small branch arising from it, in the lower part of the photograph.

Fig. 3. Original section at the level of fig. 2. The muscle of the main bronchus is seen to be continuous with the muscle of Reisseisen in its small ventro-medial branch. The pulmonary artery is seen in the section, and on the right is lung tissue, separated from the peribronchial connective tissue by a fold of pleura. Masson's trichrome stain. × 22.

Fig. 4. Transverse section of extrapulmonary bronchus of adult cat showing transitional stage in relationship between muscle and cartilage. Haematoxylin, orange-G, erythrosin. × 20.

Plate 2

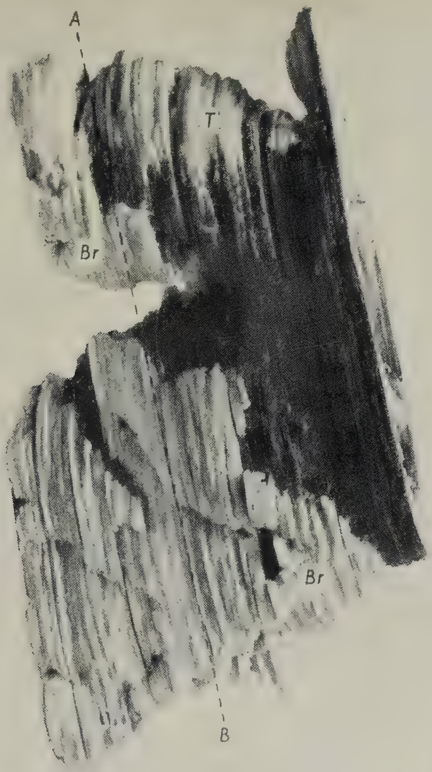
Fig. 5. Section through bronchus to right lung of kitten, showing the main bronchus cut in transverse section (to left of photograph) with the bronchus to the upper lobe arising from its lateral aspect (to right of photograph). The anterior walls of the main bronchus and of the first part of the bronchus to the upper lobe are devoid of muscle fibres. The position of the bundles of muscle fibres is indicated by arrows, and their continuity posteriorly from the one bronchus to the other is seen. The anterior wall of the upper lobe bronchus contains muscle fibres only after the establishment of a complete muscle of Reisseisen, the first indication of which is shown by the uppermost arrow. Iron haematoxylin, Van Gieson. × 28.

Fig. 6. Transverse section of bronchus to right lower lobe of the kitten (extrapulmonary portion). Masson. × 36.

Fig. 7. As fig. 6 at a level 100μ lower down the bronchus. The projection P_1 is seen to be now separated from its cartilaginous plaque (Br_1) and is therefore in the form of a hook. Masson. × 36.

Fig. 8. Transverse section of bronchus to left upper lobe of kitten. Masson. × 50.

Fig. 9. Transverse section of extrapulmonary bronchus of adult dog. The bundles of muscle fibres are arranged as in a typical muscle of Reisseisen. Haematoxylin, orange-G, erythrosin. × 16.



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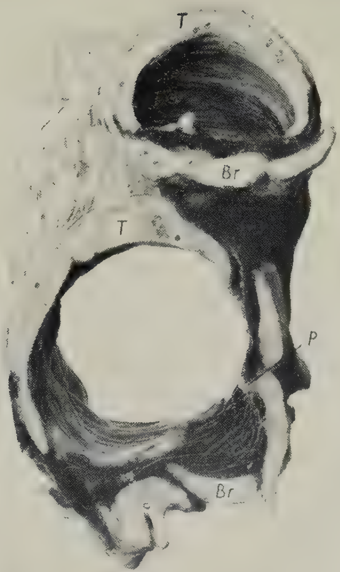

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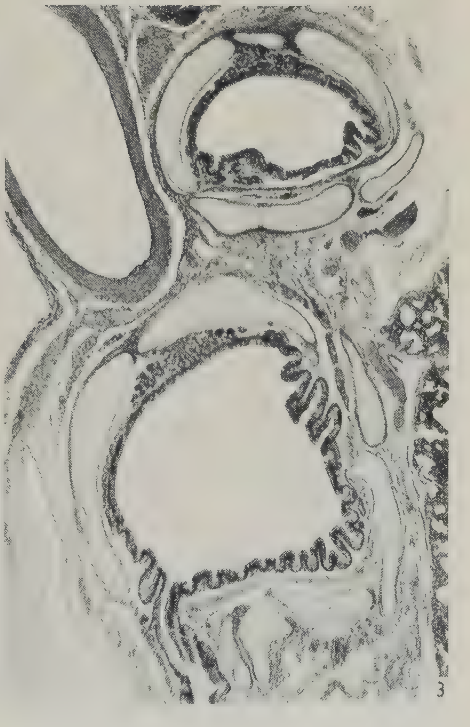

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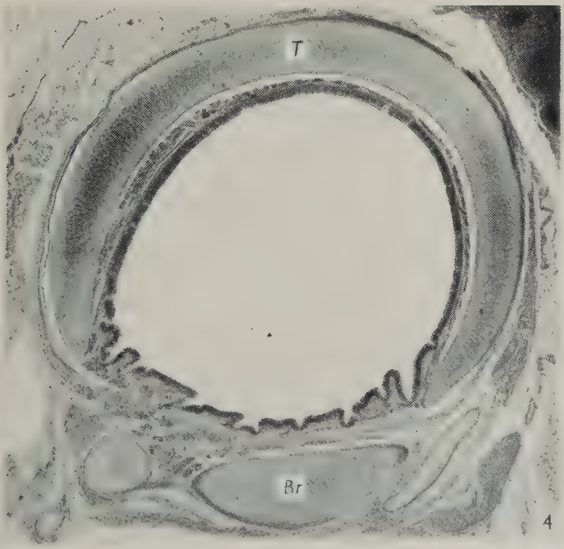

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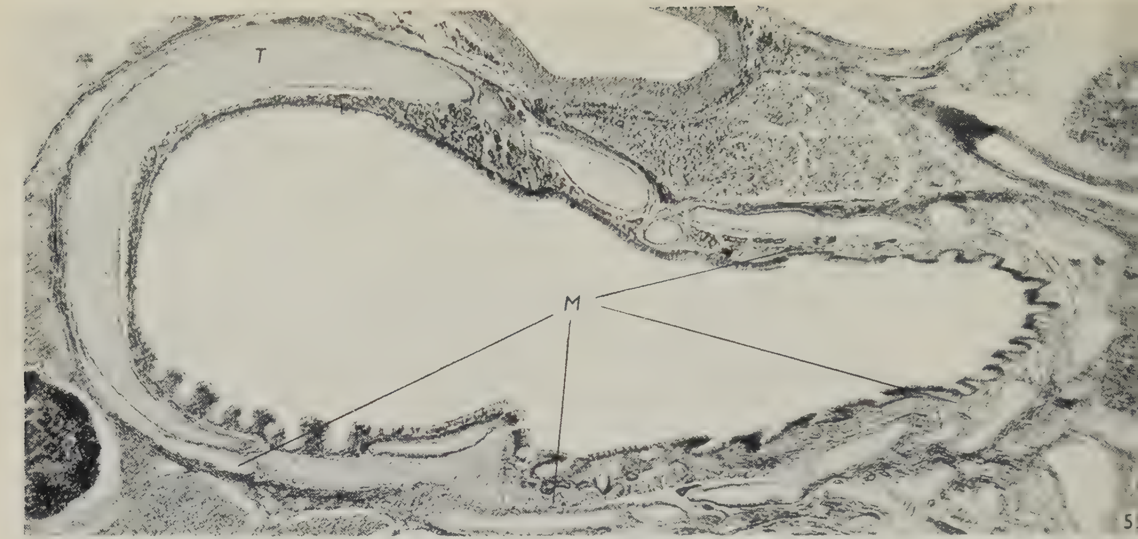
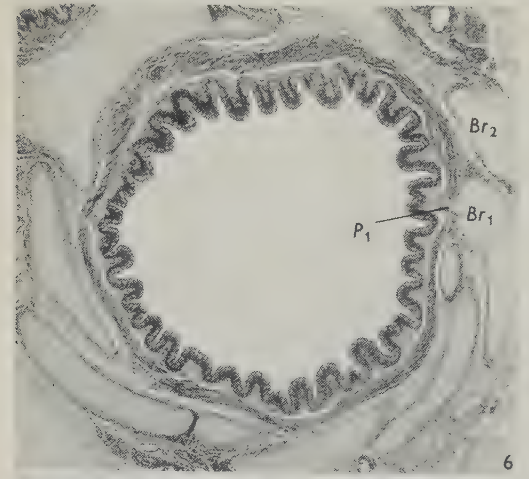
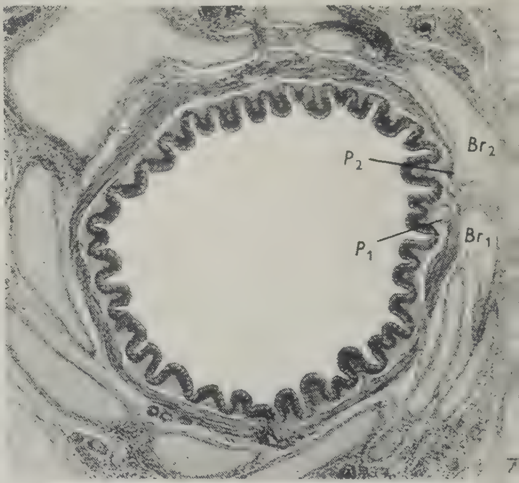
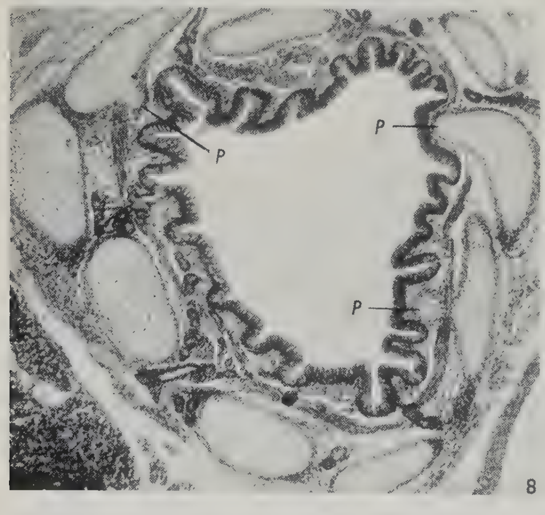
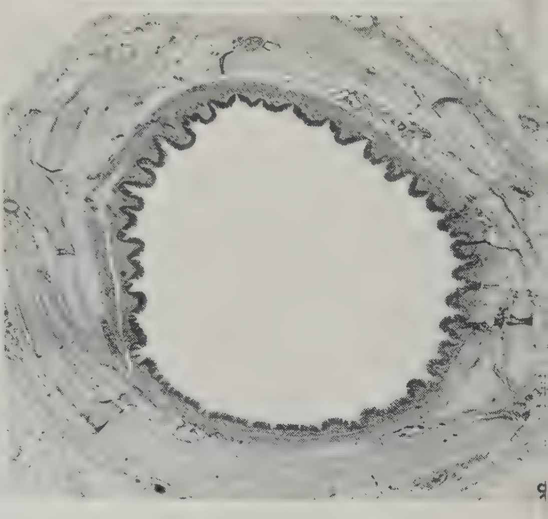
TOWERS—Muscle-cartilage relationship in the extrapulmonary bronchi











TOWERS—Muscle-cartilage relationship in the extrapulmonary bronchi

THE MECHANICS OF THE FOOT

I. THE JOINTS

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Birmingham

INTRODUCTION

The axis of the ankle joint is described in Buchanan (1949) as passing through the talus just above the talo-calcanean articulation, directly below the mid-point of the fibular malleolus and coinciding with the tip of the medial malleolus. The movement is flexion and extension, the ranges being given as 27° and 13° respectively. Gray (1949) allows slight side-to-side gliding, rotation and adduction abduction when the joint is in flexion. Steindler (1935) and Elftman & Manter (1935) give the orientation in relation to other joints, the axis being intermediate between the knee and the talo-calcaneo-navicular axes. In a recent paper Barnett & Napier (1952) have shown from a study of the shape of the articular surface that two axes must exist, one when the joint is in the flexion two-thirds of its range and the other when it is in the extension one-third. Both pass very near to the tip of the fibular malleolus, but the former slopes downwards and medially whereas the latter slopes upwards and medially (cf. Fig. 2, *p.a.* and *d.a.*).

The talo-calcaneo-navicular joint-complex evidently is difficult to comprehend. Fick (1911), modifying Donitz, stated that the movement was a simple hinge movement and that the whole of the subtaloid portion of the foot rotated about a line passing obliquely through the head of the talus (Fig. 2, *t.c.n.*). Manter (1941) re-established the direction of its axis and made an ample review of the previous literature. It is fully established that the axis runs in a downward, outward and backward direction making an angle as viewed from the side of about 45° with the horizontal and as viewed from above of about 16° with the sagittal. The work of most other authors, however, is applied to the task of interpreting this simple hinge movement in conventional anatomical and orthopaedic terms, the whole of the difficulty in comprehension arising from the oblique direction of the axis. Thus Wiles (1934) had already described 'adduction-inversion abduction-eversion' and had pointed out that the adduction abduction element, being about a vertical axis, was the same movement as external internal rotation of the leg upon the foot, the difference depending on which segment was for the time being stationary. This had also been said earlier by Lovett & Cotton (1898). Jones (1945) built a wooden model to demonstrate what he called torque in the tibia producing torque about the long axis of the foot. Levens, Inman & Blosser (1948) demonstrated the rotation of the leg in walking in the living subject by cinematography, though they did not associate their findings with the talo-calcaneo-navicular joint. Although Jones concluded that one effect of this action was to alter the distribution of weight from side to side of the foot, he found it difficult to see what purpose this served in the human.

The only other joint-complex to have received much attention is the mid-tarsal

(Chopart's) which is composed of the talo-navicular and the calcaneo-cuboid articulations. The chief point at issue has been whether or not it exists in the true meaning of a joint. Elftman & Manter described it as having an approximately transverse axis, but subsequently Manter found two movements of rotation and gave the direction of the more oblique axis as 50° to the horizontal and 60° to the sagittal planes, and of the other axis as antero-posterior but inclined 15° upward anteriorly. The motion about the first axis, it is agreed, is flexion-adduction extension-abduction. Shephard (1950), evidently referring to the antero-posterior axis, describes supination pronation. Wiles, however, denies that the joint exists at all, in that no one movement takes place there, and Inkster (1938) had previously said that such movement as occurs is insignificant.

The more anterior joints have not been clearly defined and Steindler says that they are 'rather insignificant'. Of the standard text-books Buchanan says that slight flexion extension movement takes place at the tarso-metatarsal joints. Cunningham (1951) adds that these contribute slightly to 'inversion' and 'eversion'. Wiles (1934, 1949) and Gray's Anatomy emphasize the appreciable up-and-down movement of the first metatarsal at the 'tarso-metatarsal' joint and associate this with real changes (as opposed to apparent changes) in arch height. The other metatarsals, however, Wiles suggests are closely connected by ligaments and must move in one piece. Although Steindler does not define the joints concerned he does appreciate that the metatarsal heads have some up-and-down movement relative to each other, usually reciprocal, about a comparatively fixed third metatarsal. In consequence the line across the heads of the metatarsals may assume a supinated or a pronated tilt relative to the posterior part of the foot.

The objects of the present work are, first, to show that all movements and changes in shape of the foot are rotations about axes, and secondly, to determine anew those axes already described and to establish the hitherto undescribed ones. The parts the movements play in changing the shape of the foot are discussed. Certain minor accessory movements have been omitted from consideration as they do not affect the major foot mechanisms.

The definitions of terms used in this paper are as follows: rotation about a transverse axis will be spoken of as *flexion extension*, rotation about a vertical axis when the foot is the moving part will be spoken of as *abduction adduction*, and when the leg is the moving part as *medial rotation lateral rotation*, and rotation about an anterior-posterior axis will be spoken of as *pronation supination*. The geometrical meaning of the word axis is being used, and it is necessary to point out that an axis does not necessarily pass through either of the segments concerned. Rotations about oblique axes are given compound names, e.g. *pronation-abduction-flexion supination-adduction-extension* is used for rotation about an axis which is predominantly antero-posterior but which has a moderate slope from above downward and a slight obliquity from medial to lateral, the predominant component being named first. For brevity the predominant component will sometimes be used alone, the above rotation, for example, being referred to as (modified) pronation (modified) supination, but this will only be done where the argument is not thereby invalidated. A property of a hinge joint, however, is that the rotation always remains in the same plane (see p. 352), and the abbreviated terminology must not be taken to imply that the components can ever be segregated. The term *ray* is used for a toe unit anterior to the mid-foot, i.e. cuneiform plus metatarsal in the case of the medial three and metatarsal in the case of the lateral two.

MATERIAL AND METHODS

Five normal amputated feet and ten nearly normal specimens were used for the main experiments. Only results from normals are quoted. The criteria for selection were: youth, absence of symptoms, clinical normality, radiological normality and very recent amputation. The preservation was by refrigeration, and the shape, joint position and function were kept under constant observation throughout, no deviation from the initial state being permitted.

The specimen was left entire and undissected below a point 4 cm. above the ankle. At this level tendons were picked up and attached to systems of pulleys and spring balances fixed in the positions of the leg muscles. Movements were always brought about by the natural means.

For experiments on the free foot the tibia was fixed to a support and movements were brought about by tensing or slackening the various tendons.

For experiments on the weight-bearing foot the specimen was made to stand in an apparatus (to be described in a later paper) which could apply normal body weight in normal conditions of balance controlled by the necessary tendon actions whilst allowing full freedom of leg rotation. Readings of body weight, line of body weight and tendon tensions could all be made at any moment. The apparatus always revealed when mechanically impossible or unnatural conditions were imposed. Flat standing, toe-standing and standing on sloping surfaces could all be arranged and the arch could be raised or lowered as desired.

I. *Determination of the axes*

Small spikes were driven into the bones, one proximal and one distal to each joint to be studied. A straight rod was attached to one of the spikes by malleable wire and two pointers were similarly attached to the other spike. The rod was initially arranged to lie somewhere near the supposed line of the joint-axis, and one pointer was brought close to each end (Fig. 1). Joint movement was then caused to occur.

At first both ends of the rod made excursions relative to the pointers through arcs of circles indicating that the rod was not lying true to the axis. By trial and error the rod and pointers were adjusted toward a position of minimum excursion until finally the rod made no excursion relative to the pointers but appeared to be rotating about its own long axis. The rod was now in the true axis of the joint and was fixed there by substituting a rigid wax bar for the malleable wire. It could be placed for certain within a circle not exceeding 1 cm. diameter. In this way a number of rods were held in space near the foot, each lying in the true axis of a joint and, because each was attached to its own segment, remaining true to the axis whatever movements took place. Radiographs were made of the whole arrangement from three directions.

The movement of the rod relative to the pointers was observed to be rotation in every case. Some irregularity occasionally occurred toward the extremes of movement, but it was small. Unusual forces also caused slight deviation (up to 5°) from the established line. Screw motion, i.e. shift along the axis accompanying the rotation, as described in Manter's paper, was not observed and if present could not have exceeded 2–3 mm. This experimental proof that all movements of the foot are rotations will justify the application of the laws of rotation when balance and muscle actions are studied. It is considered that none of the variations described is of sufficient magnitude to make these laws inapplicable, but it is possible that when the limitation of joint movement is studied a closer analysis of the irregularities will be required.

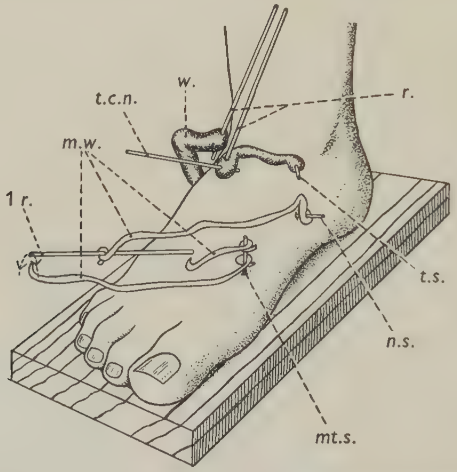


Fig. 1. Method of determining the axes. One axis has been determined and rigidly fixed to one of its segments; the other is in the process of being adjusted. The excursion of its distal end depicted would indicate that the true axis lies below the present position of the rod. *mt.s.* spike in first metatarsal carrying two malleable wire pointers; *n.s.* spike in navicular carrying axis rod for first ray joint; *t.c.n.* axis rod for talo-calcaneo-navicular joint rigidly fixed by a wax bar to spike in talus; *r.* radius rods attached to calcaneum and navicular respectively; *t.s.* spike in talus; *m.w.* malleable wire; 1 *r.* axis rod for first ray joint; *w.* wax bar coming from spike in calcaneum.

The surface markings of the axes are given in Table 1 and the directions of the axes are shown in Fig. 2.

The two main ankle-joint axes were found almost exactly in the positions predicted by Barnett & Napier (1952). The 'dorsiflexion axis'—to adopt their terminology—is the one about which rotation occurs when the joint is in the extension part of its range, and the 'plantarflexion axis' is the one about which rotation occurs in the flexion part of its range. Movement cannot occur about these two axes simultaneously.

The two mid-tarsal axes can be used simultaneously.

The three axes with which talo-navicular movement is concerned, viz. the talo-calcaneo-navicular, the oblique mid-tarsal and the antero-posterior mid-tarsal, all pass near the centre of the sphere constituted by the head of the talus.

The movements at these axes, all containing a pronation supination element, allow side-to-side swinging of the body, walking on sloping surfaces and, what is most important, the control of side-to-side balance. It has been an almost constant finding in a series of patients with severely restricted or absent talo-calcaneo-navicular movement that they were unable to maintain side-to-side balance in one-leg standing.

Table 1. *Surface markings of the axes*

Ankle joint:	
(*a*) 'Dorsiflexion axis'	1·5 cm. anterior to the tip of the medial malleolus to 0·5 cm. inferior to the tip of the lateral malleolus
(*b*) 'Plantarflexion axis'	1·5 cm. anterior and 1 cm. inferior to the tip of the medial malleolus to 0·5 cm. superior to the tip of the lateral malleolus
Talo-calcaneo-navicular joint	Supero-medial aspect of the neck of the talus to postero-lateral 'corner' of the heel
Mid-tarsal joint:	
(*a*) The 'oblique' axis	Supero-medial aspect of the head of the talus to lateral side of inferior surface of heel, 2·5 cm. anterior to the talo-calcaneo-navicular axis
(*b*) The 'antero-posterior' axis	Superior aspect of navicular about half-way between the mid-line of the dorsum of the foot and its medial border to lateral side of posterior aspect of heel, 2·5 cm. superior to the talo-calcaneo-navicular axis
First ray joint	Mid-dorsum of foot over the base of 3rd metatarsal to the tuberosity of the navicular bone
Fifth ray joint	Supero-medial border of the foot at cuneiform-first-metatarsal joint to 1·5 cm. above and behind the styloid process of the fifth metatarsal.

The angle between knee and ankle axes as viewed from above (the knee axis is approximately in the coronal plane of the diagram) should, it is suggested, be used as a measure of 'the twist of the tibia'. It is known to vary in different individuals and to alter during the first few years of life (Hutter & Scott, 1949).

II. *Measurement of range of rotation*

In addition to the artificial axis there were attached two rods, one to the proximal segment and one to the distal segment of each joint, perpendicular to the axis and therefore in the position of radii (*r*., Fig. 1). For convenience they were arranged parallel with and close to each other when the foot was in the basic position of flat standing so that they appeared superimposed when viewed along the axis. Any change in the angle between the 'fixed' and the 'moving' radius represented true joint movement and could easily be measured. Furthermore, if these rods had ceased to lie in parallel planes it could have been inferred that abnormal movement was occurring. Results are included in Table 2.

The axes remained the same whether the foot was free or weight-bearing. Abnormal movements did not occur whilst the foot was fresh.

III. *Combined ray movements*

The third ray axis was found to lie near the third cuneiform and to be transverse in direction, the movement being pure flexion extension. The second and fourth rays were not examined in detail. They were seen to behave in a manner intermediate between first and third and third and fifth respectively, and it may be presumed that the axes are intermediate. All rays have a flexion-extension component. A small bursa-like cavity was found between each pair of metatarsal heads in several specimens, presumably for the purpose of facilitating movement.

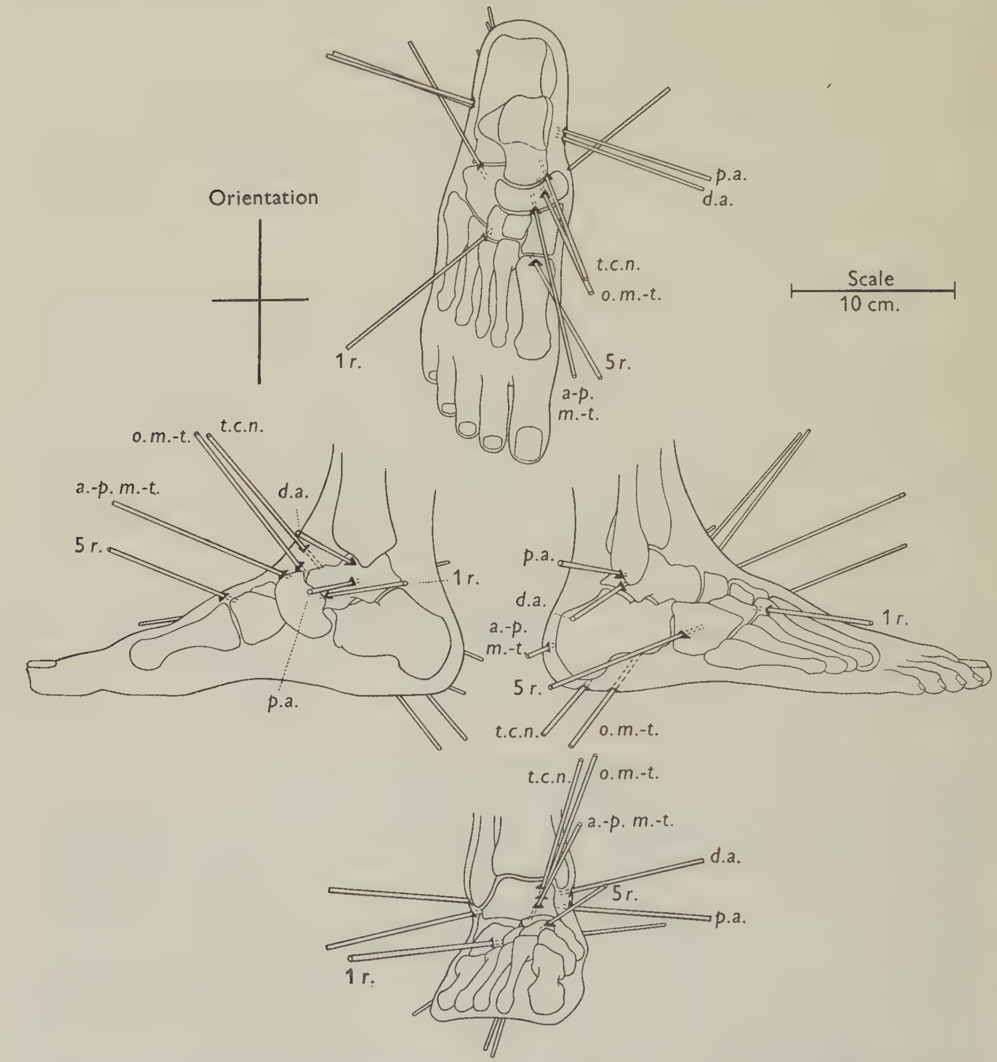


Fig. 2. The axes of all the foot joints (except those of the middle three rays); adapted from radiographs of the experimental foot. Foot in basic position of relaxed flat standing, the ankle being in a position of moderate extension, the talo-calcaneo-navicular and mid-tarsal joints at or near full pronation, the first ray at full extension-supination and the fifth ray near to full flexion-supination. *d.a.* dorsiflexion ankle axis; *p.a.* plantarflexion ankle axis; *t.c.n.* talo-calcaneo-navicular axis; *o.m.-t.* oblique mid-tarsal axis; *a.-p. m.-t.* antero-posterior mid-tarsal axis; 1 *r.* 1st ray axis; 5 *r.* 5th ray axis.

It was observed that although (modified) flexion extension is to a great degree independent for each ray it is not entirely so. The interconnexion of the rays throughout their lengths by soft tissues causes them to follow each other to some extent.

Table 2. *Movements and ranges of movement*

Joint	Description of movement	Range	Remarks
Ankle:			
(*a*) Dorsiflexion axis	Flexion with slight adduction and supination. Extension with slight abduction and pronation	50°	Work on other specimens and some normal young adults suggests that a more usual range is about 12° greater
(*b*) Plantarflexion axis	Flexion with slight supination. Extension with slight pronation		
Talo-calcaneo-navicular	Pronation-abduction-extension. Supination-adduction-flexion	24°	Other specimens and other methods show agreement to within 3°
Mid-tarsal:			
(*a*) The oblique axis	Pronation-abduction-extension. Supination-adduction-flexion	22°	
(*b*) The antero-posterior axis	Pronation with slight abduction-extension. Supination with slight adduction-flexion	8°	
First ray	Flexion-pronation. Extension-supination	22°	Other specimens and living subjects show agreement to within 8°
Third ray	Flexion. Extension	10°	
Fifth ray	Flexion-supination. Extension-pronation	10°	

Note. These data are quoted from one selected specimen. The last column shows what variations may be expected.

Extreme flexion of the first ray causes moderate flexion of the second, and the same applies to the fifth and fourth rays.

A combined movement which very readily occurs is reciprocal movement of medial and lateral rays, flexion of the first being accompanied by extension of the fifth and vice versa with the intermediate rays taking up intermediate positions. This results in a twist of the anterior part of the foot (Fig. 3). It is not a simple motion because the shape of the forefoot is not constant but changing and therefore there is no true axis. However, for convenience an axis is assigned to it along the relatively motionless third ray. Pronation twist is said to occur when the first ray flexes and the fifth extends and supination twist when the first extends and the fifth flexes. This can be imitated for easy visualization by the four fingers of one's hand.

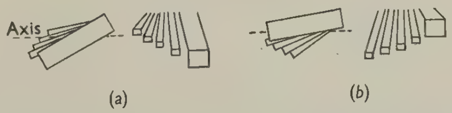


Fig. 3. Positions of the metatarsals in forefoot twist. (*a*) pronation twist; (*b*) supination twist.

IV. *Individual joint movements and the shape of the foot*

The foot was made to assume certain of the positions commonly occurring in life, and the positions of the individual joints were observed from the radius rods. The results are given in Table 3. The arch raising was brought about by passively extending the big toe (Hicks, 1951).

Table 3. *Change in foot shape*

	Observed positions of individual joints			
	Talo-calcaneo-navicular	Mid-tarsal	First ray	Fifth ray
Foot free:				
'Inversion', i.e. sole of foot directed medially	Supination-adduction-flexion	Supination	Extension-supination	Flexion-supination
			i.e. supination twist of forefoot	
'Eversion', i.e. sole of foot directed laterally	Pronation-abduction-extension	Pronation	Flexion-pronation	Extension-pronation
			i.e. pronation twist of forefoot	
Foot standing:				
Arch high	Supination-adduction-flexion	Supination	Flexion-pronation	Extension-pronation
			i.e. pronation twist of forefoot	
Arch low	Pronation-abduction-extension	Pronation	Extension-supination	Flexion-supination
			i.e. supination twist of forefoot	

The terms 'inversion and eversion' are used variously by different authorities, for while some (e.g. Quain, Cunningham) apply them to the combined movements at several joints others (e.g. Fick, Jones, Manter) use them to mean rotation about an antero-posterior axis, and others again (e.g. Gray) restrict their use to the movement which occurs at the talo-calcaneo-navicular joint.

DISCUSSION

Mechanical laws, if correctly applied, are absolute. The whole of the functions of weight-bearing, walking, maintenance of balance, etc., can be explained mechanically. Definition of the axes is an essential preliminary.

Plane of movement predetermined

The joints all being hinge joints their plane of movement is predetermined. Unlike a ball-and-socket joint whose plane of movement is determined by the directions of the forces acting across it, each foot joint has its plane of movement predetermined by its own intrinsic structure. The plane is perpendicular to the axis. Different tendons, though they may run in oblique directions, always produce one of two movements, clockwise or anticlockwise rotation about the axis. This holds true also for other forces such as body weight. For example, a force which tends to produce abduction at the talo-calcaneo-navicular joint only succeeds in producing pronation-abduction-extension because this (or its opposite) is the only movement of which the joint is capable.

The mid-tarsal joint

Sufficient evidence has been presented to show that Wiles's (1934, 1949) contention that there is no true joint at the mid-tarsal region is incorrect, nor is the movement insignificant as has been stated by Inkster. It can readily be demonstrated in the living foot if the examiner, having first ensured that all muscles are relaxed, passively manipulates the forefoot (approximately) from side to side. The calcaneum must, of course, be held firmly by the other hand, but although the total movement is thereby limited (because the talo-calcaneo-navicular joint is being fixed) there still remains one-third to one-half of the full range of 'inversion eversion'.

The talo-navicular articulation being of ball-and-socket shape might be expected to show three degrees of freedom, and this may seem incompatible with the assertion that all the joints are hinge joints. This joint is, however, peculiar in being one-half of two different joint-complexes. In conjunction with the talo-calcanean articulations it forms the talo-calcaneo-navicular joint-complex. In conjunction with the calcaneo-cuboid articulation it forms the mid-tarsal joint-complex. The navicular therefore rotates on the talus about three different axes—about the talo-calcaneo-navicular axis when the talo-calcaneo-navicular complex is in action and about one or other of the mid-tarsal axes when the mid-tarsal complex is in action. The motion remains a simple hinge movement in each case. Often, of course, action at both of these complexes occurs simultaneously. A spherical surface for the articulation between talus and navicular is one way of enabling all these hinge movements to take place.

The effect of forefoot twist in the standing foot

The movements described in Table 3 as occurring together are interdependent when standing. The following explanation shows why this is so.

In ordinary standing the foot comes to rest with the sole flat on the ground, i.e. the heel and the metatarsal heads all lie in one horizontal plane.

If a foot that has its forefoot twisted into pronation as in Fig. 3*a* (p. 351) is put to the ground the medial side of the ball of the foot will touch down first whilst the lateral side is still clear. One of three things must then happen before a stable stance is reached. The twist of the forefoot may undo, i.e. the first ray may extend and the fifth flex until the metatarsals heads all come to lie level with each other. Alternatively, supposing that the twist in the forefoot will not undo—in the next paper it will be shown how this may come about—the entire forefoot unit may supinate on the posterior part of the foot at the mid-tarsal joint until the pronation tilt of the line across the metatarsal heads is neutralized. Alternatively, if neither the pronation of the forefoot will untwist nor the mid-tarsal joint supinate, the only remaining way in which the line across the metatarsal heads can come to lie horizontal is for the entire foot to tilt over in a supination direction. The foot would then stand as depicted in Fig. 4*a*. The arched hand resting on a table is a good aid to visualization, the twist of the forefoot being imitated by appropriate movements of the fingers. Table 3, giving the results of direct observations, shows that in fact both the two latter alternatives take place, viz. supination occurs both at the mid-tarsal and at the talo-calcaneo-navicular joints. On the other hand, when the forefoot assumes

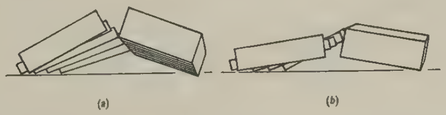


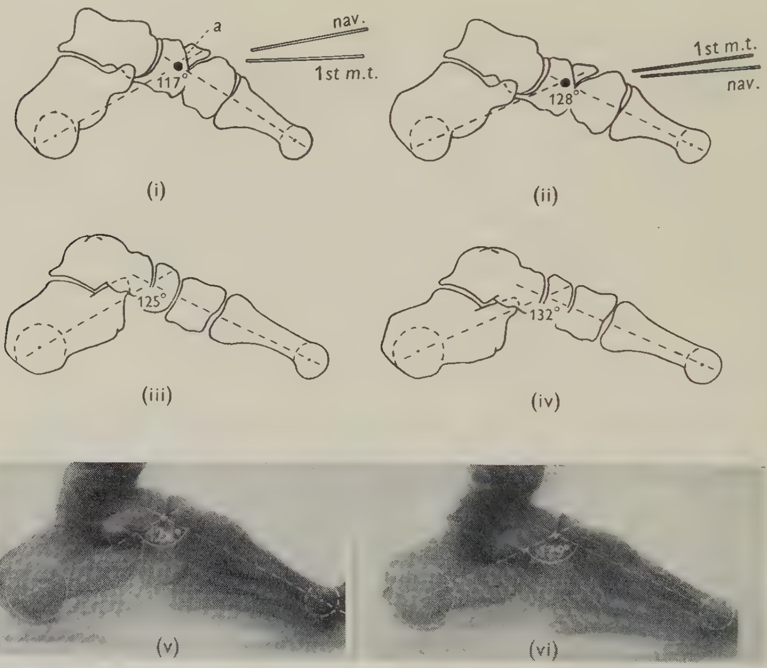
Fig. 4. Showing how forefoot twist influences the position in pronation supination of the posterior part of the foot.

a supination twist as in Fig. 3*b*, then pronation must occur at the mid-tarsal joint or at the talo-calcaneo-navicular joint or at both, and the foot takes up the position shown in Fig. 4*b* when placed on the ground. Analogy with a three-legged stool, two legs anterior and one posterior, may be helpful, the tilt of the stool as it stands on the ground being determined by the relative lengths of the two anterior legs. The effect is also seen in Fig. 6.

This description, which implies that twist of the forefoot may be primary and that the whole foot may be tilted into supination or pronation secondarily, is intentional. It is wrong to assume that it is always the leg muscles acting from the proximal aspect which determine the shape of the foot. It would be equally wrong, on the other hand, to assume that they never do.

The shape of the arch

Referring again to Fig. 4 it will be seen that besides representing the positions in pronation supination of the various parts of the foot the model also appears to show changes in arch height.



(i) (ii)

(iii) (iv)

(v) (vi)

Fig. 5. Showing that arch raising is flexion of the ray. *a.* axis rod (end view); *nav.* radius rod attached to navicular; 1st *m.t.* radius rod attached to first metatarsal.

Wiles has gone some way toward distinguishing between apparent and real changes in arch height. Fig. 5 shows that real changes in arch height are nothing other than flexion and extension of the ray. Diagrams (i) and (ii) are traced from radiographs of an experimental foot viewed along the axis of the 1st ray joint.

In (i) the ray has been flexed, whereas in (ii) it has been allowed to extend. The angular movement is demonstrated by the change in position of the radius rods (and measures 11° in this case). Diagrams (iii) and (iv) are traced from radiographs taken at the same time but along a different line, viz. the latero-medial line which is more practicable for radiography in the living. (The angular movement now appears to be only 7°, a discrepancy due to the distortion of a changed viewpoint.) Diagrams (v) and (vi) are similar latero-medial radiographs but of a living foot in the high-arch and low-arch positions respectively. Both the experimental and the living foot were weight-bearing, and the change from ray extension to ray flexion (i.e. from low arch to high arch) was brought about by the same means, viz. by extending the metatarso-phalangeal joint. Thus it is demonstrated that ray flexion is the same movement as arch rising, the only difference being the custom of using the former term when the foot is free and the latter when it is standing. It is true therefore to say that the movements that go to make up pronation twist of the forefoot are raising of the medial arch and flattening of the lateral arch.

Talo-calcaneo-navicular movement

Whereas the mid-tarsal joint provides for rotation between anterior and posterior parts of the foot, the talo-calcaneo-navicular joint provides for rotation between foot and leg. In other respects the oblique mid-tarsal and talo-calcaneo-navicular axes are so similar that they may be visualized as one, the oblique hinge.

If when standing the arch is raised it is a matter of common observation that the leg laterally rotates. This same rotation about a vertical axis is also to be seen in one-leg balancing; when the foot tends to supinate to correct a tendency to overbalance laterally, the leg rotates laterally, and when the foot tends to pronate, the leg rotates medially. Each instance is an example of the invariable association of supination of the foot with lateral rotation of the leg (and vice versa) noted by Lovett & Cotton.

This apparently double movement, as has been shown by Jones, is nothing other than simple hinge movement at the talo-calcaneo-navicular joint. It is difficult to visualize because the hinge is oblique, but the model illustrated in Fig. 6 will help. The posterior part of the foot is shown supinated in Fig. 6 (1) and it will be seen that the 'tibia' is in a position of lateral rotation relative to it. In Fig. 6 (2) the posterior part of the foot has become pronated, and it is seen that the tibia has rotated medially simultaneously. The movements are a simple rotation about the peg which represents the axis. These movements, to be seen both in the living and in the experimental specimen, have been identified with talo-calcaneo-navicular movement by the experiments described above. Furthermore, in the standing specimen the lateral rotation of the tibia was seen to occur when the posterior part of the foot supinated as a result of pronation twist of the forefoot (see p. 353), this in turn being brought about by raising the medial arch. By this chain of actions it comes about that the femur laterally rotates at the hip joint when the big toe is lifted off the ground.

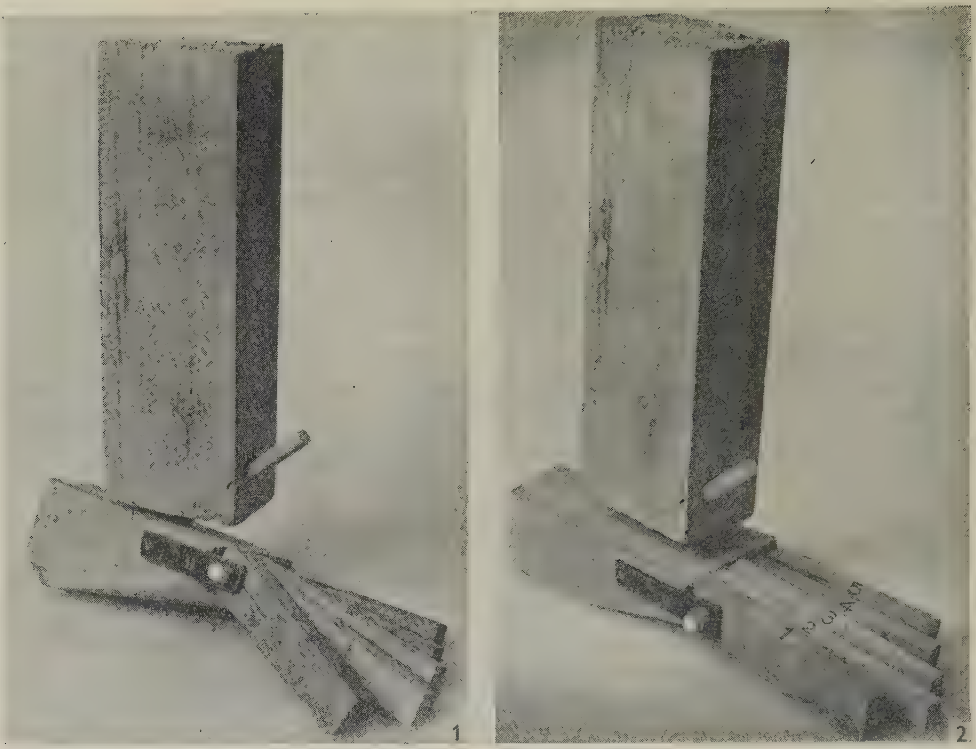


Fig. 6. Showing that supination of the posterior part of the foot and lateral rotation of the leg (and vice versa) are manifestations of the same simple hinge movement. (The talo-calcaneo-navicular axis is correctly orientated but the ray axes are not (cf. Fig. 2), the transverse alignment of the latter being a simplification which conforms with the abbreviated terminology of 'flexion', etc., used in the text for 'flexion-pronation', etc. This approximation does not invalidate the argument in so far as it is taken in this paper, but one neat effect of the true axes is that the first and fifth metatarsal heads remain almost square with the ground instead of riding over on to their lateral corners as they do here.)

SUMMARY

Experiments are described on mechanically normal amputated legs to show that all movements in the foot are rotations. The sites at which these rotations take place are defined as joints. It is demonstrated that in natural conditions rotations occur at the ankle (two axes), the talo-calcaneo-navicular, the mid-tarsal (two axes) and at the five ray joints. (The toes are not included.) Surface markings and geometrical data of orientation and range of movement are given from a typical foot.

When the foot is free the movements at these joints can be summarized as follows: the whole foot undergoes flexion extension upon the fixed leg (the flexion is accompanied by slight supination and the extension by slight pronation); the part distal to the talus in addition undergoes pronation-abduction-extension supination-adduction-flexion; the part distal to the mid-tarsal joint in addition undergoes (approximate) pronation supination; the forepart of the foot in addition undergoes a pronation-supination twist.

The conditions of standing impose certain restrictions, viz. a constant relationship between the plane of the metatarsal heads (horizontal) and the tibia (near vertical), so that any movement at one of the intervening joints must be neutralized by an opposite movement at another. The necessary association of rising of the arch, pronation twist of the forefoot, supination at the oblique hinge and lateral rotation of the leg has been explained, and it has been shown that these or their converse must and do occur together.

In one-leg standing side-to-side balance is maintained by movement at the talo-calcaneo-navicular joint. The rotation at the oblique axis of this joint is in this circumstance manifested as simultaneous movement of leg and foot, lateral rotation of the leg accompanying supination of the foot and vice versa. In the absence of talo-calcaneo-navicular action these movements do not occur; also it is found that one-leg balancing is impossible.

REFERENCES

Barnett, C. H. & Napier, J. R. (1952). The axis of rotation at the ankle joint in man. *J. Anat., Lond.*, **86**, 1–9.

Buchanan, A. M. (1949). *Buchanan's Manual of Anatomy*, 8th ed., edited by F. Wood Jones. London: Baillière, Tindall and Cox.

Cunningham, D. J. (1951). *Cunningham's Textbook of Anatomy*, 9th ed., edited by J. C. Brash. London: Oxford University Press.

Elftman, H. & Manter, J. (1935). The evolution of the human foot, with special reference to the joints. *J. Anat., Lond.*, **70**, 56–67.

Gray, H. (1949), *Gray's Anatomy*, 30th ed., edited by T. B. Johnston & J. Whillis. London: Longmans, Green and Co.

Fick, R. (1911). *Handbuch der Anatomie und Mechanik der Gelenke*, Abt. 1, Teil 3; incorporated in *Handbuch der Anatomie*, edited by Bardeleben, **2**, 612–626. Jena: Gustav Fischer.

Hicks, J. H. (1951). The function of the plantar aponeurosis. *J. Anat., Lond.*, **85**, 414–415.

Hutter, C. G. & Scott, W. (1949). Tibial torsion. *J. Bone Jt. Surg.* **31**-A, 511–518.

Inkster, R. G. (1938). Inversion and eversion of the foot and the transverse tarsal joint. *J. Anat., Lond.*, **72**, 612–613.

Jones, R. L. (1945). The functional significance of the declination of the axis of the subtalar joint. *Anat. Rec.* **93**, 151–159.

Levens, A. S., Inman, V. T. & Blosser, J. A. (1948). Transverse rotation of the segments of the lower extremity in locomotion. *J. Bone. Jt. Surg.* **30**-A, 859–872.

Lovett, R. W. & Cotton, F. J. (1898). Some practical points in the anatomy of the foot. *Boston med. surg. J.* **139**, 101–107.

Manter, J. T. (1941). Movements of the subtalar and transverse tarsal joints. *Anat. Rec.* **80**, 397–410.

Shephard, E. (1950). The movements of the tarsus. *J. Bone Jt. Surg.* **32**-B, 283.

Steindler, A. (1935). *Mechanics of Normal and Pathological Locomotion in Man.* London: Baillière, Tindall and Cox.

Wiles, P. (1934). Flat-feet. *Lancet*, **127**, 1089–1094.

Wiles, P. (1949). *Essentials of Orthopaedics.* London: J. and A. Churchill.

JUGUM CEREBELLARE INTERSEMILUNARE

By JELENA KRMPOTIĆ-BOHAČEK

The writer, in two previous publications (1948, 1951), has referred briefly to the existence of a bony ridge in the cerebellar fossa of the occipital bone; the present paper gives an account of the shape, variations and significance of this formation.

The ridge does not appear to be described or mentioned in anatomical texts, though it figures in many anatomical illustrations. Frazer (1933 and 1937) mentions 'transverse striations' in the cerebellar fossa, but these cannot be identified with the ridge under discussion. Goldhammer & Schüller (1927), recounting variations in the radiological appearance of the occipital bone, describe one example of a ridge which corresponds with that described here, but make no remark on its significance.

The text-books of Gray (1944) and Morris (1946) illustrate accurately a well-marked ridge, while others (Loder, 1803; Oesterreicher, 1852; von Spee, 1896; Toldt, 1940; Chiarugi, 1936; Sobotta, 1919; Quain, 1893; Woerdeman, 1948; and others), illustrate a slightly developed ridge, but none of them mention it in the text.

In the present investigation 866 occipital bones have been examined. The series consists of 674 intact skulls, 103 disarticulated skulls ranging in age from 1 year to adult life, and 89 isolated occipital bones. The age limits of the whole series are from 1 to 77 years.

In addition, 221 cerebella have been examined with special regard to the features on the occipital surface of the hemispheres.

An oblique ridge in the cerebellar fossa of the occipital bone runs from the inferior margin of the transverse groove towards the mastoid emissary foramen (Fig. 1). The ridge separates two shallow depressions: an upper one which lodges the superior semilunar lobule ('impressio lobuli semilunaris superioris') and a lower one for the inferior semilunar lobule ('impressio lobuli semilunaris inferioris'). As the ridge corresponds with the fissure separating the two semilunar lobules, the name 'Jugum cerebellare intersemilunare' is suggested for it.

The distance of the medial end of the ridge from the internal occipital crest varies from 5 to 50 mm., the average value being 20 mm. The distance of the lateral end of the ridge from the inferior margin of the transverse groove varies from 5 to 25 mm., the average distance being 14 mm.

The intersemilunar ridge is not always well marked, and it is not always developed in its whole length (Fig. 2). The formation varies from a well-marked ridge through all transitions to complete absence. In the 866 specimens (1732 halves) it was absent on the right in 170 (19·63 %) and on the left in 166 (19·17 %). The ridge should be inspected under appropriate illumination and it is advisable, in addition, to palpate it with the fingertips.

In the 80 % of cases in which the ridge was present, its degree of development varied as recorded in Table 1 (see Fig. 2).

Measurements of the cerebellum have been made for comparison with those of the occipital bone. The height of the superior semilunar lobule varies from 10 to

25 mm. The distance of the medial end of the horizontal fissure (i.e. the point at which it passes over from the inferior to the superior cerebellar surface) from the medial margin of the hemisphere varies from 0 to 30 mm. That is to say, the horizontal fissure may terminate on the medial margin itself.

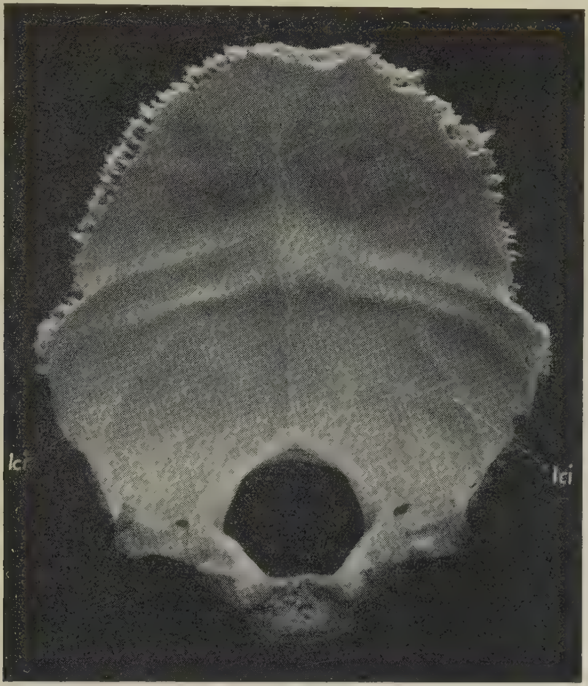


Fig. 1. The occipital bone of a 9-year-old girl with well-marked jugum cerebellare intersemilunare (*Ici*).

Table 1. *Incidence of variations in the types of the ridge*

Type of ridge	Right no.	%	Left no.	%
Continuous	446	51·50	438	50·57
Interrupted	6	0·69	8	0·92
Medially and centrally	2	0·23	2	0·23
Laterally and centrally	4	0·46	5	0·58
Medially and laterally	1	0·12	4	0·46
Medially only	36	4·16	42	4·85
Laterally only	154	17·78	168	19·39
Centrally only	47	5·42	33	3·81
Totals		80·71		80·83

(The different types of ridge are illustrated in Fig. 2.)

Comparison of the 442 cerebellar measurements with those of the occipital bone naturally show some lack of agreement, for the reason that the most medial part of the horizontal fissure is not related to the occipital bone but to the inferior layer of the dura mater of the transverse sinus.

The ridge on the bone can be correlated with the horizontal fissure of the cerebellum, for the other deep fissures of the cerebellum leave no impression on the bone. The reason lies in the fact that folio limiting the other fissures on the inferior cerebellar surface run mostly parallel with the fissures, while in the case of the horizontal fissure the folia run obliquely into its depth, as described by Ziehen (1903). The lips of the horizontal fissure are thereby rounded off and separated from each other. The oblique course of the folia at the margins of the horizontal fissure is variable, so that the horizontal fissure may be deep or shallow and produce a corresponding imprint in the cerebellar fossa of the occipital bone.

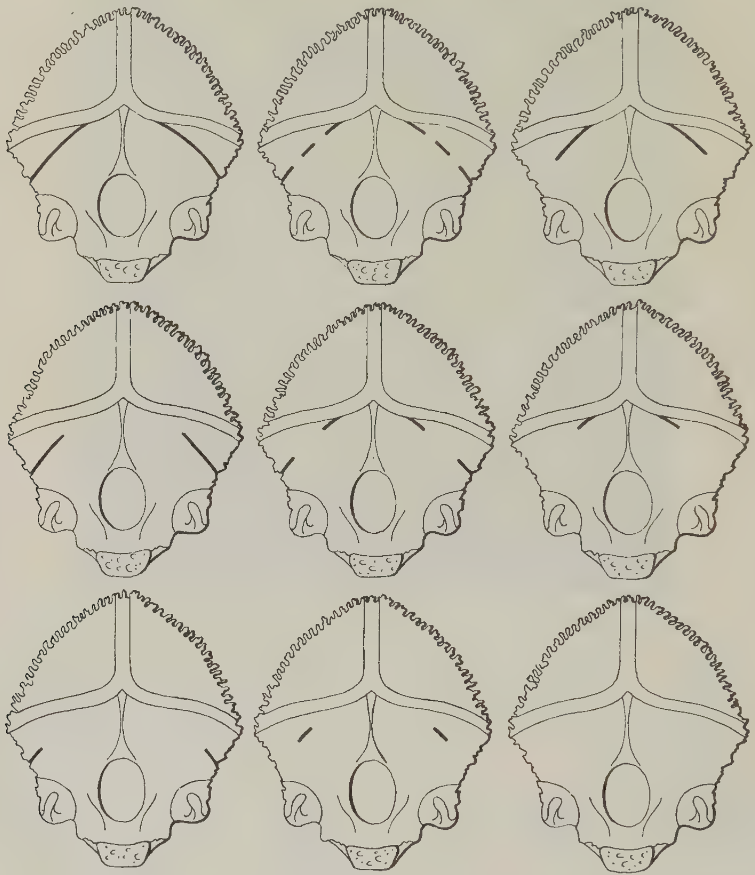


Fig. 2. Different shapes of the jugum cerebellare intersemilunare.

REFERENCES

Chiarugi, G. (1936). *Instituzione di anatomia dell'uomo*, 4th ed., vol. **1**. Milan: Società editrice libraria.

Frazer, J. E. (1933). *The Anatomy of the Human Skeleton*, 3rd ed. London: J. and A. Churchill.

Frazer, J. E. (ed.) (1937). *Buchanan's Manual of Anatomy*, 2nd ed. London: Baillière, Tindall and Cox.

Goldhammer, K. & Schüller, A. (1927). Varietäten im Bereiche der hinteren Schädelgrube. (Mit besonderer Berücksichtigung der operativen Eingriffe in dieser Gegend.) *Fortschr. Röntgenstr.* **35**, 1163–1190.

Gray, H. (1944). *Anatomy: Descriptive and Applied*, 28th ed. reprint. Ed. by T. B. Johnston and J. Whillis. London: Longmans, Green and Co.

Krmpotić-Bohaček, J. (1948). Studies concerning the formerly unknown impressions of the cerebellar folia in the inferior fossae of the occipital bone in children. *Bull. int. Acad. yougosl. Sci.* (N.S.), **1**, 55–60.

Krmpotić-Bohaček, J. (1951). Studies concerning the spiral lobules in the human cerebellum. *Bull. int. Acad. yougosl. Sci.* (N.S.), **3**, 125–161.

Loder, J. Ch. (1803). *Anatomische Tafeln zur Beförder der Kenntniss des menschlichen Körpers.* (Issued in 4 vols. 1794–1804.) Weimar: Landes Industrie Comptair.

Morris, Henry (1946). *Morris' Human Anatomy*, 10th ed. Ed. by J. P. Schaeffer. Philadelphia: Blakiston Company.

Oesterreicher, H. (1852). *Anatomischer Atlas oder bildliche Darstellung des menschlichen Körpers*, 2nd ed. München: J. Palm's Hofbuchhandlung.

Quain, J. (1893). *Elements of Anatomy*, 10th ed. Ed. by E. A. Schäfer and G. D. Thane. London Longmans, Green and Co.

Sobotta, J. (1919). *Atlas der deskriptiven Anatomie des Menschen*, 3rd. ed. München: J F. Lehmanns Verlag.

Spee, F. Graf von (1896). Skelettlehre. 2. Kopf. In Bardeleben's *Handbuch der Anatomie des Menschen*, Bd. 1, Abt. 2. Jena: Fischer.

Toldt, C. (1940). *Anatomischer Atlas für Studierende und Ärtze*, 18th ed., Bd. 1. Ed. by F. Hochstetter. Berlin und Wien: Urban und Schwarzenberg.

Woerdeman, M. W. (1948). *Atlas of Human Anatomy*, vol. 1. Baltimore: Williams and Wilkins.

Ziehen, Th. (1903). Centralnervensystem. In Bardeleben's *Handbuch der Anatomie des Menschen*, Bd. 4, Abt. 1. Jena: Fischer.

AN INVESTIGATION OF THE ORIGIN OF THE COLOSTRUM CELLS*

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There are conflicting views on the origin of the cellular elements of the colostrum. The opinion most commonly held is that the large and small colostrum cells originate from mesenchyme and enter the colostrum by diapedesis through the epithelium of the breast alveoli and ducts. This view was supported, among other authors, by Bizzozero & Vassale (1887) and Varrier-Jones (1924). On the other hand, Wallich & Levaditi (1905) held that both wandering cells and epithelial cells may be present in the colostrum. Grégoire (1930), among others, goes further, and suggests that the majority of the cells are epithelial in origin.

There are very few histological data on the changes in the epithelium of the human breast between late pregnancy and the early post-parturitional stages. Most of such investigations of the colostrum cells have been carried out on lactating animals such as the guinea-pig and cat.

The present investigation deals especially with the histological changes in the human breast in the early post-parturitional stage, and supports the view that the majority of the colostrum cells are epithelial in origin.

MATERIAL AND METHOD

Sections from fifty-eight breasts were examined. Forty-five of these came from women who died in the first few days after delivery, three came from women who died during parturition and ten others in the resting stage or in different menstrual phases were used for comparison. The ages of the subjects varied from 12 to 40 years.

All the breasts were fixed in 10% formalin and the sections stained with haematoxylin and eosin. Sagittal sections of the whole breast were cut at a thickness of 100μ. With such sections it was possible to compare different areas in the same breast and also to obtain a three-dimensional picture of the glands. Some thin sections (10μ) were used to obtain a clear picture of the cellular structure, but thin sections did not give such a complete idea of the structure in depth and in them the glandular elements were seen in disconnected fragments. Smear preparations of colostrum were also examined.

RESULTS

The resting breast. There are so many factors affecting the histology of the adult breast that separate descriptions are needed of the different phases.

Breasts of young women who died of chronic diseases, especially tuberculosis, were chosen. In cases of this kind, menstruation usually ceases and the mammary

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gland can be assumed to be in the resting stage. Figs. 1 and 2 show characteristic features of what may be classified as the resting breast. Histological examination showed clusters of alveoli at the periphery (Pl. 1, fig. 1), whereas ducts with few alveoli were characteristic of the centre of the corpus mammae (Pl. 1, fig. 2).

There were many variations in the glandular elements, not only in the relative abundance of glandular elements in different breasts, but also in the relative abundance and degree of cellular differentiation within a section of the same breast. The varying amounts of glandular tissue in the breasts of different women has been described in a previous paper (Engel, 1941), but the striking variations in different parts of one and the same breast are now emphasized by means of whole sections of breasts used in this investigation. This variation in alveolar structure is best shown by studying different sites in the same section of a whole breast. Pl. 1, fig. 3, shows a section of the whole breast from a girl aged 17 years. The arrows show the sites from which the photographs in Pl. 1, figs. 4 and 5, and Pl. 2, fig. 6, were taken. Well-marked ducts with few budding alveoli are seen in Pl. 1, fig. 4. The alveolar pattern has become more complex in Pl. 1, fig. 5; marked proliferation of the alveoli is shown in Pl. 2, fig. 6. This extensive proliferation was most frequently seen at the basal periphery, and may be sufficiently well marked as to give an appearance of changes due to pregnancy.

The epithelium lining the budding alveoli in these non-lactating breasts has a double layer of cells. The external cells are small and inconspicuous in the resting breast (Pl. 3, fig. 14) but become much more distinct as soon as the breast is under increased hormonal stimulation (Pl. 2, fig. 10). The two layers are distinct from the outer myoepithelium which does not directly concern this investigation. The generally accepted view that there is only one layer of cells lining the alveoli cannot be confirmed.

The breast during menstruation

Detailed histological changes during menstruation were described by Rosenburg (1922). There is no general agreement, but proliferation is believed to occur during the first phase of the cycle, followed by regression in the second phase. Dieckmann (1925) is of the opinion that the mammary gland does not respond at all in the menstrual cycle. The correlation of clinical experience with the findings of this investigation suggests that the response depends on the proportion of the glandular and fibrous content.

The proliferation of the alveoli during the first phase of the menstrual cycle is shown in Pl. 2, fig. 7, and in fig. 8 the two layers of cells are more marked than in the resting breast. The outer or basal layer of cells consists of large, pale cells with small nuclei. The cytoplasm has been shown to contain neither glycogen nor fat droplets. The internal layer is composed of small, closely packed cells, almost filled by the nucleus, hence their dark staining.

Chronic mastitis

Chronic mastitis, or mastopathia, occurs in many women, particularly in those over 35 years of age. It is now generally accepted that the condition is not inflammatory but due to hormonal imbalance. Many cases show the effect of hormonal stimulation on the secretory basal cells. Pl. 2, fig. 10, shows the large, clear basal cells and the smaller and darker internal layer from such a case. The dark nuclei of the inner layer make a sharp contrast with the pale outer layer.

The histological changes in the breast during parturition

Three of the breasts examined were from women who died during delivery and were therefore in the colostral stage. The specimens were most useful in the elucidation of the different nature of the two cell layers. Pl. 2, fig. 9, and Pl. 3, fig. 11, show alveoli from two of these cases. Pl. 2, fig. 9, shows alveoli now lined with a single layer of cells and containing desquamated cells in the lumen that have probably been shed from the internal epithelial layer. Pl. 3, fig. 11, shows, in one alveolus, a complete internal layer coming away from an intact basal layer. In examination of many sections of these breasts all stages have been seen, from the intact internal layer still partly adherent to the basal layer, that is, in the act of desquamation, to the debris in the lumen of an alveolus now lined with a single layer of epithelium. This debris can be seen collected in the small alveolar ducts illustrated in Pl. 3, fig. 13, which shows a small alveolar duct packed with cellular debris, although its own epithelial lining is intact.

Some indication of the fate of the duct epithelium is given by Pl. 3, fig. 16. This part of the wall of a glandular duct shows epithelial proliferation and hydropic degeneration. These cells are large, their nucleus is small and the pale cytoplasm contains neither glycogen nor fat droplets demonstrable by Best's glycogen stain or Sharlach R. These hydropic cells are very similar to the large colostrum cells. It is possible that some of the desquamated cells retain fat droplets or fat may be ingested after desquamation.

A colostrum smear (Pl. 3, fig. 15) illustrates a typical large colostral cell probably derived from hydropic duct cells and short chains and coherent clusters of small darkly staining cells. Floating freely in the colostrum, the cells are spherical, and it is suggested that they come from the internal epithelial layer of the alveoli.

Wandering cells abound in the tissues supporting the alveoli, but only a few polymorphonuclear cells were seen in the colostral smears.

DISCUSSION

The fact that the alveoli of the non-lactating breast are lined by two rows of epithelium is stressed because it bears on the origin of the small colostral cells. The present position in regard to the origin of the colostral cells can best be summed up by quoting Saner (1950): '...cells from the periductal stroma reach the alveolar lumen by passing through the basement membrane; these entering cells are chiefly lymphoid, but there are also numerous eosinophil cells, plasma cells, monocytes and polymorphonuclear leucocytes. Many of these cells contain fatty debris.'

The evidence presented here does not support the above statement, but suggests that the small colostral cells are in fact derived from the internal layer of the double layer of cells lining the alveoli, in the non-lactating breast, and that this inner layer of cells is shed into the lumen at the time of onset of parturition. The majority of the so-called lymphocytes are probably of this epithelial origin. Occasional polymorphonuclear cells can easily be recognized, but other members of the family of wandering cells could not be distinguished with any certainty.

The alveoli of the lactating breast are lined with a single layer of cells. The material examined indicates that in non-pregnant women the alveoli have two layers of epithelium. In the resting stage the basal layer is inconspicuous but under increased hormonal stimulation, as in some breasts during menstruation, and in chronic mastitis, the two layers become more clearly defined.

The two layers have already been shown by Berka (1912) and Dawson (1934). In Dawson's paper, fig. 15 shows the two layers of cells, and her fig. 26 on p. 672 shows the 'vacuolated' basal cells in a breast during the intermenstrual phase. Dawson is quite definite about the two epithelial layers in the alveoli of the non-lactating breasts. No explanation of the difference and of the loss of the one layer in pregnancy is brought forward. Berka is also definite about the two layers of epithelium. His description is not quite clear as he maintains that the non-lactating breast only contains ducts. His illustrations, however, show typical glandular clusters situated in loose connective tissue contrasting to the dense fibrous tissue of the mammary body.

As regards the large colostral cells, the position presents itself as follows: the idea that these cells are chiefly or exclusively wandering cells has never been convincingly proved. The fat laden colostrum cells in the writer's preparations were so similar to the proliferating cells in the ducts that these hydropic cells may well be the forerunners of the large colostrum cells. Both these cells are characterized by their large cytoplasm and small nucleus. Whether they contain fat debris or not is dependent on their past history.

The so-called colostrum bodies are probably small agglomerations of fat droplets and not cells (Forsell, 1939).

The conclusion arrived at is that the majority of the colostrum cells is of epithelial origin; wandering cells form only a small minority. This conclusion is particularly supported by the examination of material from parturient women.

SUMMARY

1. The alveoli of the non-lactating mammary gland are lined by a double row of cells. The basal layer is best recognized when the breast is under increased hormonal stimulation.

2. The basal layer is composed of the secretory cells, whereas the internal layer protects the former and is cast off as soon as the breast functions. The cast-off cells of the internal layer appear as small colostrum cells.

3. The large colostrum cells take their origin from proliferated and degenerated cells of the ducts. It is concluded that the majority of colostrum cells is epithelial in nature; a small minority belongs to the family of wandering cells.

REFERENCES

Berka, F. (1912). Zur histologischen Charakteristik der fibro epithelialen Mammatumoren. *Beitr. path. Anat.* **53**, 284–323.

Bizzozero, G. & Vassale, G. (1887). Über die Erzeugung und die physiologische Regeneration der Drüsenzellen bei den Säugetieren. *Virchows Arch.* **110**, 155–214.

DAWSON, EDITH K. (1934). A histological study of the normal mamma in relation to tumour growth. *Edinb. med. J.* **41**, 653–682.

DIECKMANN, H. (1925). Über die Histologie der Brustdrüse bei gestörtem und ungestörtem Menstruationsablauf. *Virchows Arch.* **256**, 321–356.

ENGEL, S. (1941). Anatomy of the lactating breast. *Brit. J. Child. Dis.* **38**, 14–21.

FORSELL, P. (1939). Über die Colostrum-Körperchen. *Mschr. Kinderheilk.* **77**, 215–227.

GRÉGOIRE, C. (1930). Nature épithéliale de certains corpuscles de colostrum. *C.R. soc. Biol., Paris*, **104**, 1308–1310.

ROSENBURG, A. (1922). Über menstruelle durch das Corpus Luteum bedingte Mammaveränderungen. *Frankf. Z. Path.* **27**, 466–506.

SANER, F. D. (1950). *The Breast.* Bristol: John Wright and Sons Ltd.

VARRIER-JONES, P. C. (1924). The cellular content of milk. *Lancet*, **2**, 537–542.

WALLICH, V. & LEVADITI, C. (1905). Sur la nature des éléments cellulaires du colostrum. *Ann. Inst. Pasteur*, **19**, 321–334.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Virgin aged 19 years, died of nephritis. Breast shows several lobules of glandular tissue with well-marked budding alveoli. The picture is taken from the basal periphery. × 26.

Fig. 2. Virgin, aged 40 years. The breast shows a few ducts with poorly developed alveoli. × 100.

Fig. 3. Contact print of section from which figs. 4–6 are taken. The places from which figs. 4–6 are taken are marked by arrows.

PLATE 2

Figs. 4–6. Various places of one and the same section (fig. 3), showing primitive (fig. 4), more developed (fig. 5) and greatly proliferated glands (fig. 6). × 90. (Figs. 4 and 5 are on Pl. 1.)

Figs. 7, 8. Virgin, aged 19 years. Effect of beginning hormonal stimulation (proliferative phase of the cycle). Enlargement of the external layer of cells (fig. 8) (× 245); lobular proliferation (fig. 7) (× 70).

Fig. 9. Woman died intra partum, aged 26 years. There are many desquamated cells in the alveoli, the external (secreting) layer being intact. × 200.

Fig. 10. Mastopathia (biopsy) showing the two rows of cells lining the alveoli. × 160.

PLATE 3

Fig. 11. Woman died in delivery. Desquamated but coherent internal cellular layer (arrow). × 180.

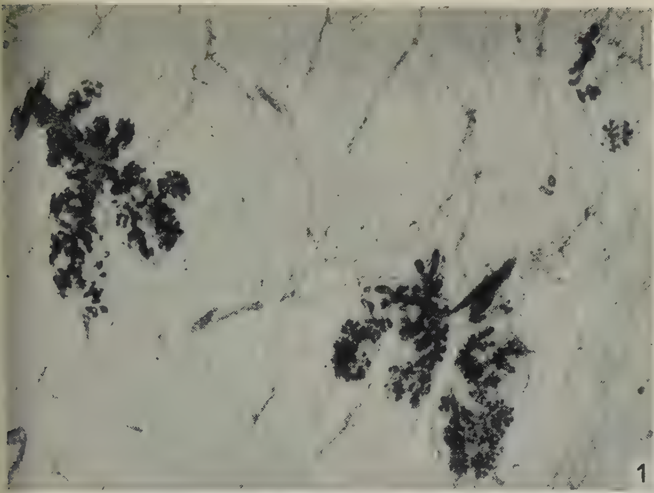
Fig. 12. Distended alveoli in fibrous portion of a colostral breast. Woman died in delivery. × 135.

Fig. 13. Woman died intra partum. Duct contains many desquamated cells while its lining is intact. × 220.

Fig. 14. Beginning alveolar proliferation to show the basal cells. × 180.

Fig. 15. Colostral smear showing rows and clusters of small cells. The cell in the centre is vacuolated, indicating the position of fat droplets dissolved out during fixation. × 250.

Fig. 16. Woman died intra partum. Proliferated and degenerated cells originating from the epithelium of a duct. × 260.



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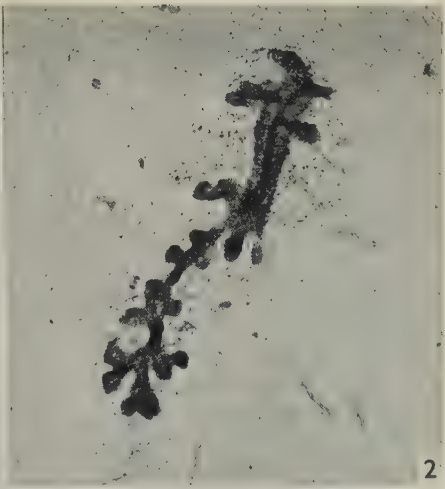

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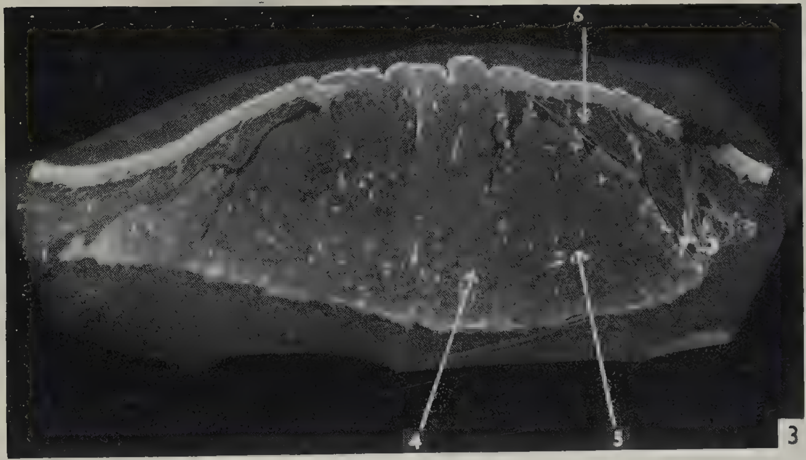

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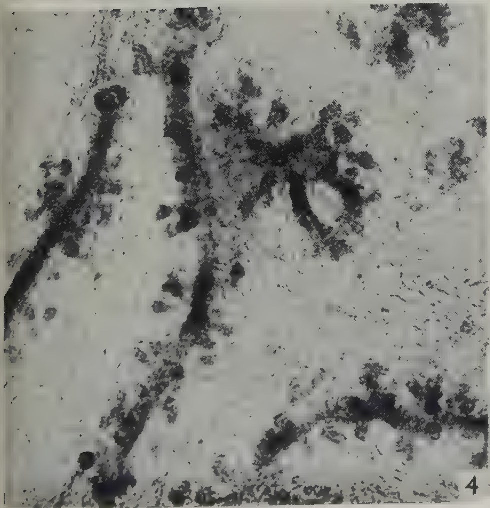

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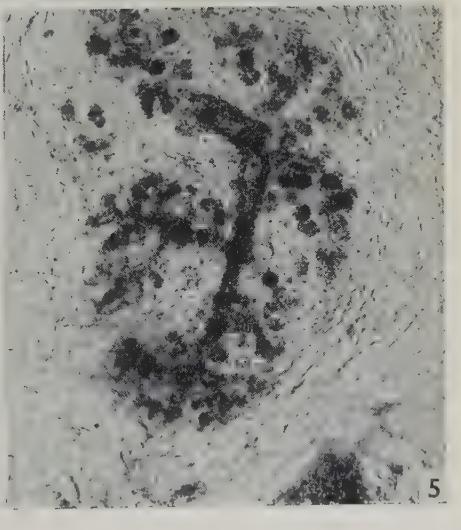

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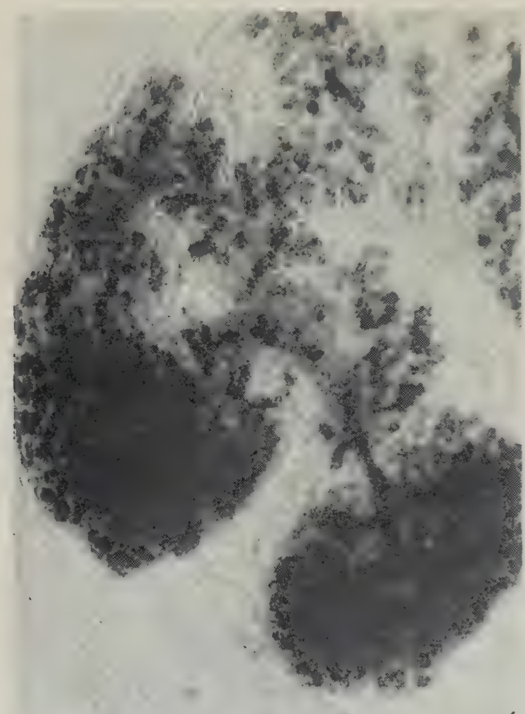
ENGEL—An investigation of the origin of the colostrum cells



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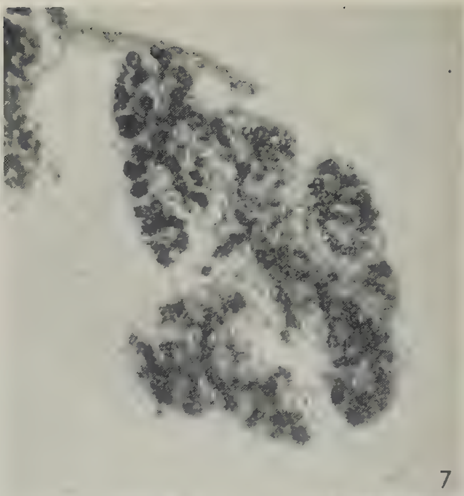

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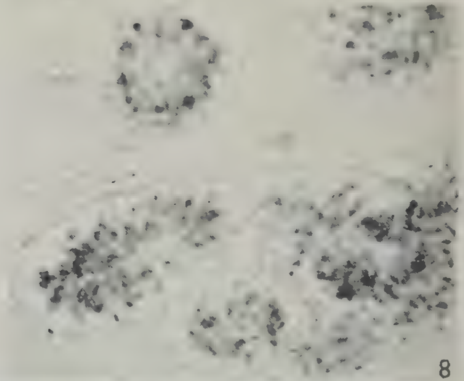

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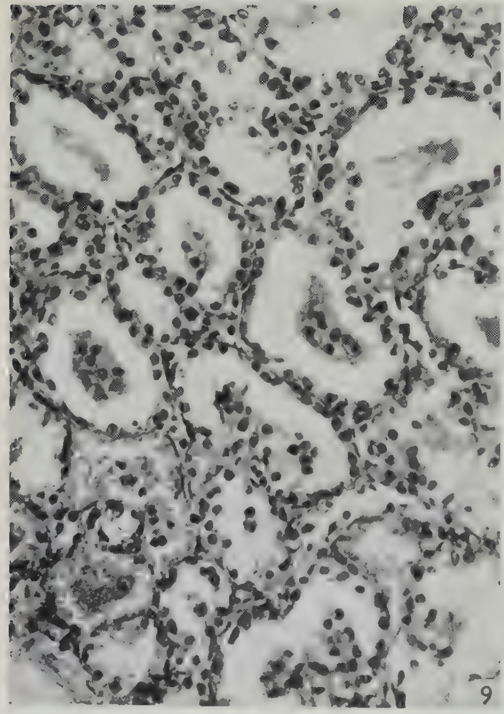

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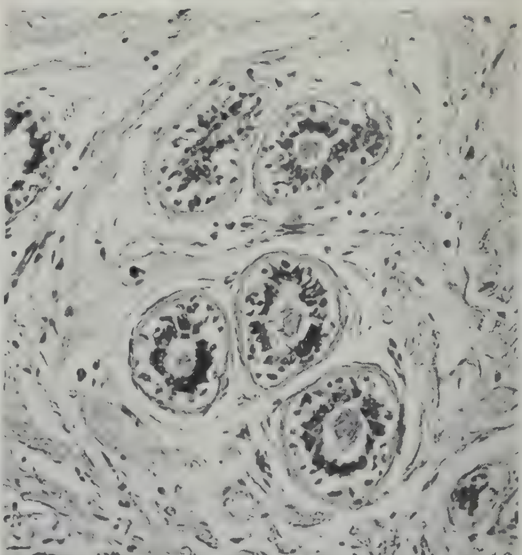

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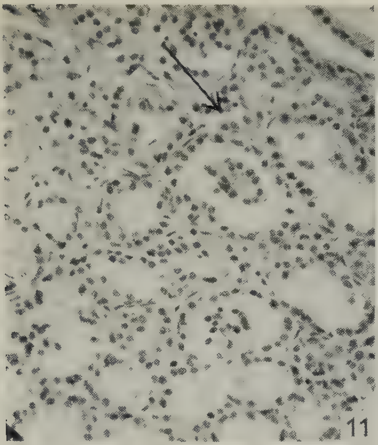
ENGEL—AN INVESTIGATION OF THE ORIGIN OF THE COLOSTRUM CELLS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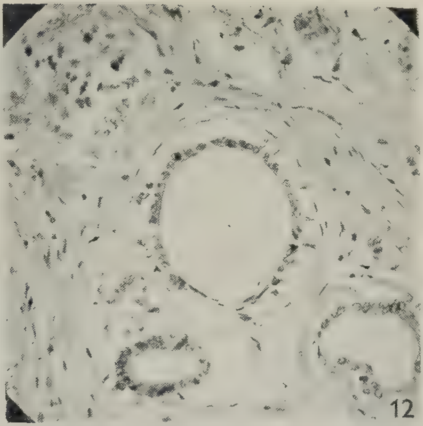
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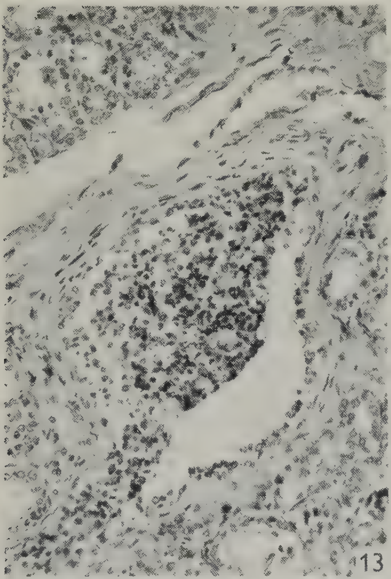
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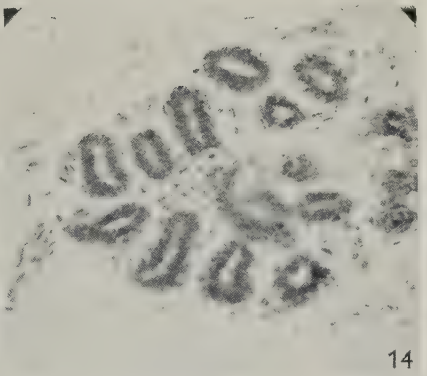
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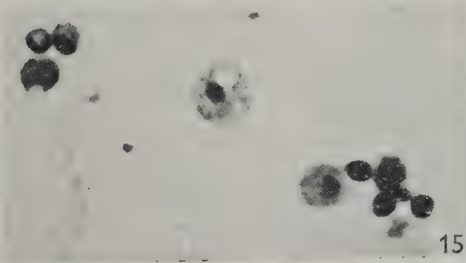
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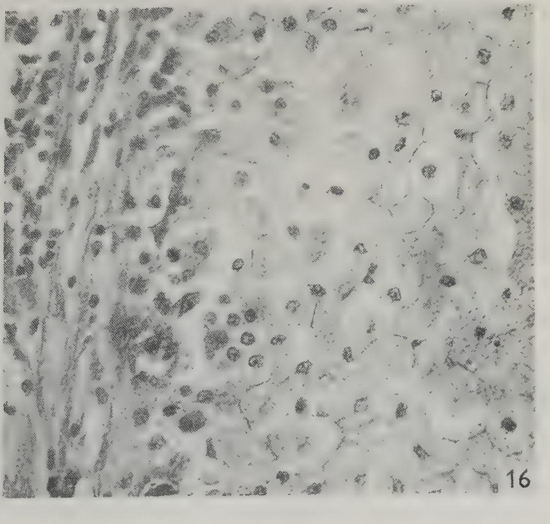
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ENGEL—AN INVESTIGATION OF THE ORIGIN OF THE COLOSTRUM CELLS

OBSERVATIONS ON THE NARIAL MUSCULATURE OF CROCODILIA AND ITS INNERVATION FROM THE SYMPATHETIC SYSTEM

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INTRODUCTION

The Crocodilia possess a number of adaptive modifications which involve the structures associated with the anterior part of the respiratory tract, and which are not found in other water-living reptiles. These modifications include the very extensive development of the bony palate, the posterior position of the internal nares, the presence of valvular flaps of tissue at the back of the throat which can shut off the mouth from the nasopharynx, and the occurrence of a special mechanism for opening and closing the nostrils. Such features make it possible for the animal to submerge its head without flooding either the nasal cavities or the more posterior regions of the respiratory pathway, even when the jaws are open under water, as when prey is being seized.

The occurrence in crocodiles of a special system of muscles associated with the nostrils has long been known. Hoffmann (1890) noted that the muscle fibres are of unstriped character; Röse (1893) figured them in transverse section without comment in a paper dealing with the nasal glands. Bruner (1897) gave a brief preliminary account of these muscles without illustration, while Bertau (1935) described them more fully and worked out their mode of action, but figured them only in transverse section. We have been able to amplify Bertau's account in certain particulars and to describe the remarkable manner in which these muscles receive their innervation, a problem which does not appear to have been previously investigated.

The following observations are based mainly on sectioned embryonic and juvenile material of *Alligator mississippiensis* and *Caiman latirostris*, while sectioned specimens of *Crocodilus niloticus* and *C. porosus* were also examined. The movements of the nostrils in life were studied in several juvenile crocodiles and caimans.

THE NARIAL MUSCULATURE

The nostrils of crocodiles open at the summit of an elevation situated on the dorsal aspect of the snout (Pl. 1, figs. 1, 2). They are usually maintained in a half open position when the animal is on land or resting quietly in the water with only the narial elevation and the orbits exposed above the surface.

In the *Crocodilus* species studied the nostrils were found to open directly upwards on to the dorsal aspect of the snout and are with their associated muscles very close to the midline. In *Alligator* and *Caiman* the nostrils are directed more laterally and together with the narial muscles lie further apart. The nostrils also have a more oblique position in relation to the long axis of the head than in *Crocodilus*.

Apart from these slight differences, which may perhaps be correlated with the width of the snout, the condition of the nostrils and narial muscles is very similar in all the Crocodilia examined. Our account of the muscles and their mode of action agrees essentially with the observations of Bruner (1897) and Bertau (1935).

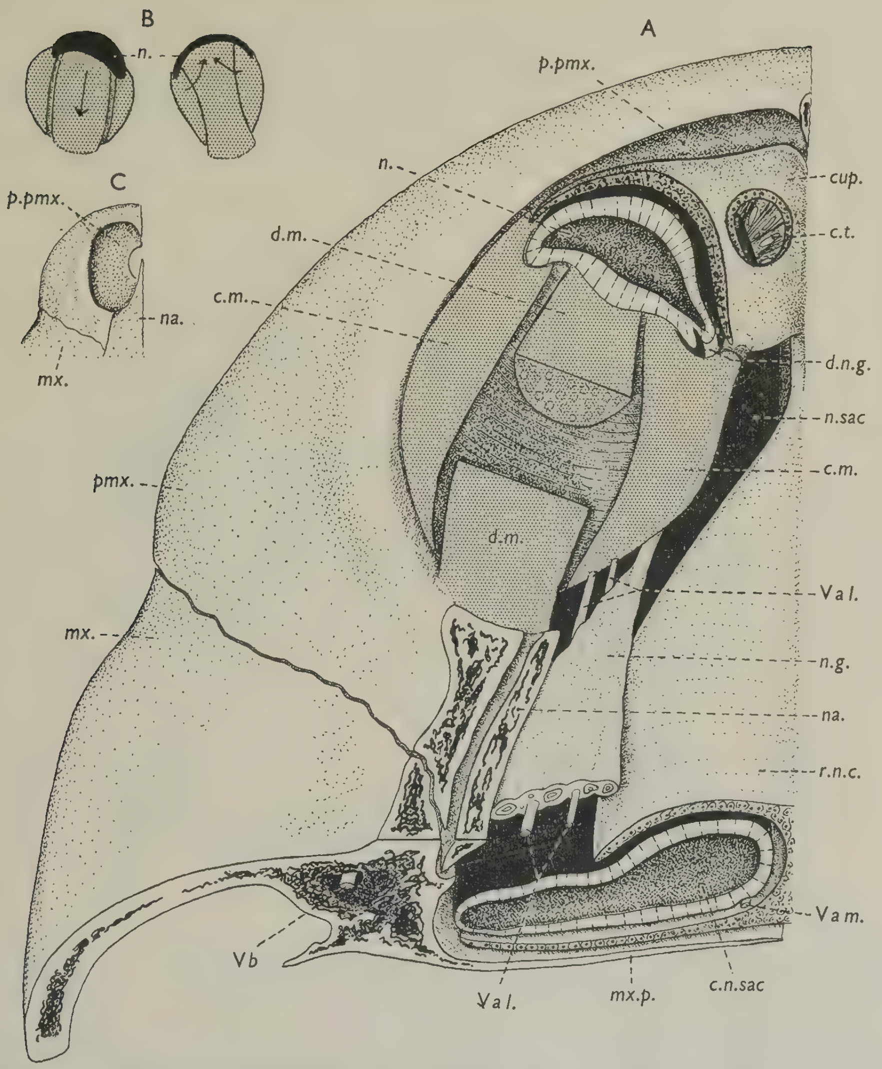
The main narial musculature (Text-figs. 1–3; Pl. 1, figs. 4, 6 and 7) is placed immediately behind the opening of each nostril and makes up the bulk of the narial elevation on each side. Ventrally it fills up the posterior part of the fossa on each side of the bony snout between the premaxilla and the nasal bone (Text-fig. 1C). In accordance with previous observations the individual muscle fibres were found to be unstriped in character.

Each set of muscles consists essentially of a complete ring or sleeve of circular fibres, termed by Bruner (1897) the *musculus constrictor naris*, through which passes a stout bundle of longitudinal fibres, the *m. dilator naris*. The latter is attached posteriorly to the premaxilla (not to the maxilla as Bertau states) and nasal bones at the back margin of the premaxillary fossa, and anteriorly to the thickened epithelium of the middle part of the posterior narial wall. The circular muscle does not seem to be attached to the surrounding bones, although a few of its posterior fibres approach the nasal. The great majority of its fibres pursue a circular course and form a sleeve around the longitudinal muscle (Text-fig. 3; Pl. 1, fig. 4). Anteriorly, however, the circular muscle becomes more complicated and its fibres pass forwards on each side of the nostril as lateral and medial bundles which are attached to the margins of the narial opening. A few fibres from each of these bundles run further anteriorly and become inserted into the free edge of the cartilage of the nasal cupola in front of the nostril (Text-figs. 1A, 2). Of the two bundles the medial is the larger and lies mainly dorsal to the duct of the nasal gland, most of its fibres running parallel with the duct. The latter, after passing through the substance of the medial bundle of circular muscle, opens into the inner angle of the nostril.

It is possible to deduce the mode of action of the two main components of the narial musculature from the conditions described (see Text-fig. 1B). The inner longitudinal or dilator muscle appears to act simply by drawing back the posterior wall of the nostril, thus enlarging the aperture. Contraction of its fibres will cause the muscle as a whole to become bunched up into a plug widest across the middle of its length; this can only take place if the outer circular muscle fibres are relaxed.

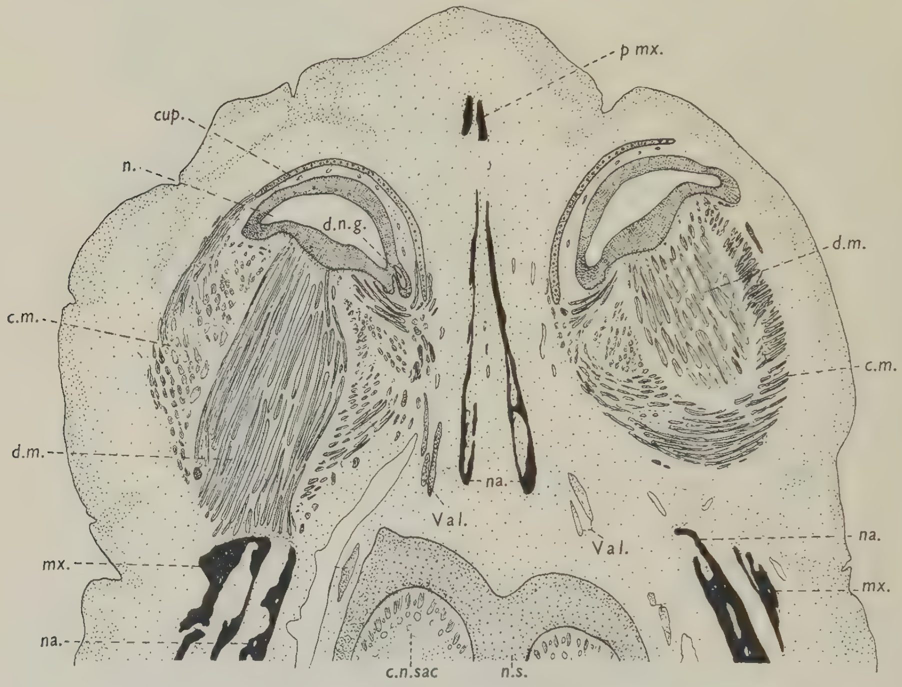
The circular constrictor muscle has a more indirect action. It appears to close the nostril mainly by squeezing the middle of the relaxed longitudinal muscle so that the front of the latter is pushed against the posterior narial wall like a piston, apposing it to the anterior wall. At the same time the attachment of the anterior circular fibres to the margins of the nostril and the cupola will serve to anchor the constrictor muscle in front and ensure that the pressure which it exerts through the anterior part of the longitudinal muscle will be concentrated against the back of the nostril. The antagonistic mode of action of the dilator and constrictor muscles was deduced by Bertau from the fact, which we have also observed, that in dead crocodiles the nostrils are always partly open. This suggested to him the presence of separate opening and closing muscles, both in a state of rigor.

Examination of living crocodilians confirms the previous observations. When the nostril is fully dilated the aperture is almost circular. Closure takes place entirely by forward movement of the posterior wall, converting the aperture into an oblique crescent which narrows until the posterior wall is in contact with the anterior wall



Text-fig. 1. A, diagrammatic reconstruction showing narial muscles and related structures in *Alligator* seen from above and behind. Parts of the premaxilla, maxilla and most of the nasal have been cut away by a horizontal section. The section has also passed through the nostril and its muscles, removing the dorsal fibres of the constrictor which conceal the dilator, and the dorsal half of the dilator itself. A segment of the latter muscle has also been removed in the transverse plane to show the ventral fibres of the constrictor. A circular window has been cut in the cartilage of the nasal cupola to show the cavernous tissue. Cut surfaces of muscles are shown in machine stipple, intact outer surface of nasal sac in black. B, diagram showing mode of action of the muscles on the nostril which is half open on the left, nearly closed on the right. In actual specimens the two nostrils are always open to the same extent. The muscles are shown in horizontal section. C, dorsal surface of intact bones of left side of snout in young *Alligator*; ×1.

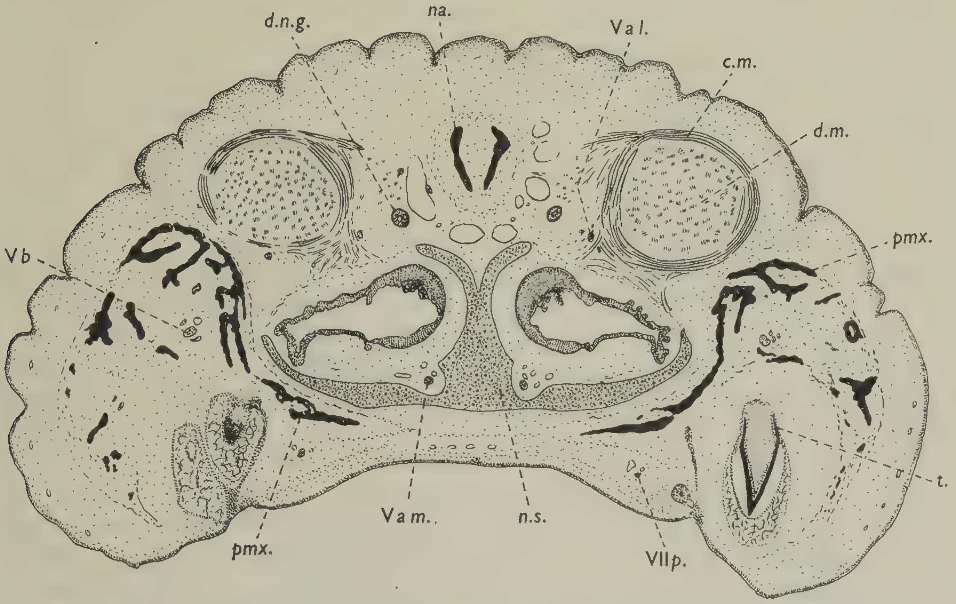
all along its length. No movement of the anterior wall was seen to occur during this process. The narial elevation behind the nostril can be seen to vary in height during movement of the nostril; it becomes more prominent when the latter is opened and flattened when it is closed, presumably conforming with the changes in shape of the underlying musculature. In all the specimens examined the state of contraction or dilatation of the nostrils was the same on each side and movements involved both sides equally. These movements can be quite rapid, as when the animal suddenly ducks its head under the water.



Text-fig. 2. *Alligator*, juvenile. Horizontal section through snout. × 20.

Study of the snout region in embryonic crocodilians shows that the narial muscles develop *in situ* from condensations of mesenchyme behind the nostrils (Pl. 1, fig. 3). In embryos of *Alligator miss.* the muscles of the nostrils are first recognizable around the 12 mm. head-length stage and are considerably later in their appearance than the striated branchial musculature of the jaws.

The epithelium of the external nares of crocodiles is of stratified type and is greatly thickened to form anterior and posterior pads which exclude the entry of water when they are forced into apposition by the muscles. The deeper layers of the epithelium contain a very large number of dendritic pigment cells (Pl. 1, fig. 5), whereas the mucosa of the main nasal sac is not pigmented. Between the anterior part of the nasal sac and the cartilage of the cupola there is a system of wide vascular channels separated by strands of smooth muscle which are quite distinct from the main narial musculature previously described. These supplementary muscle strands were also noted by Bertau. With their accompanying vessels they form a tissue comparable with the cavernous tissue which is found in relation to the front parts of the nasal sac in other reptiles. In crocodiles this tissue may play some part in altering the size of the lumen of the anterior regions of the nasal sac. Possibly it also serves a thermoregulatory function by allowing exchange of heat between the inspired air and the varying amounts of blood admitted into the cavernous spaces.



Text-fig. 3. *Alligator*, 26 mm. head-length embryo. Transverse section through snout showing narial muscles cut about halfway along their length. ×25.

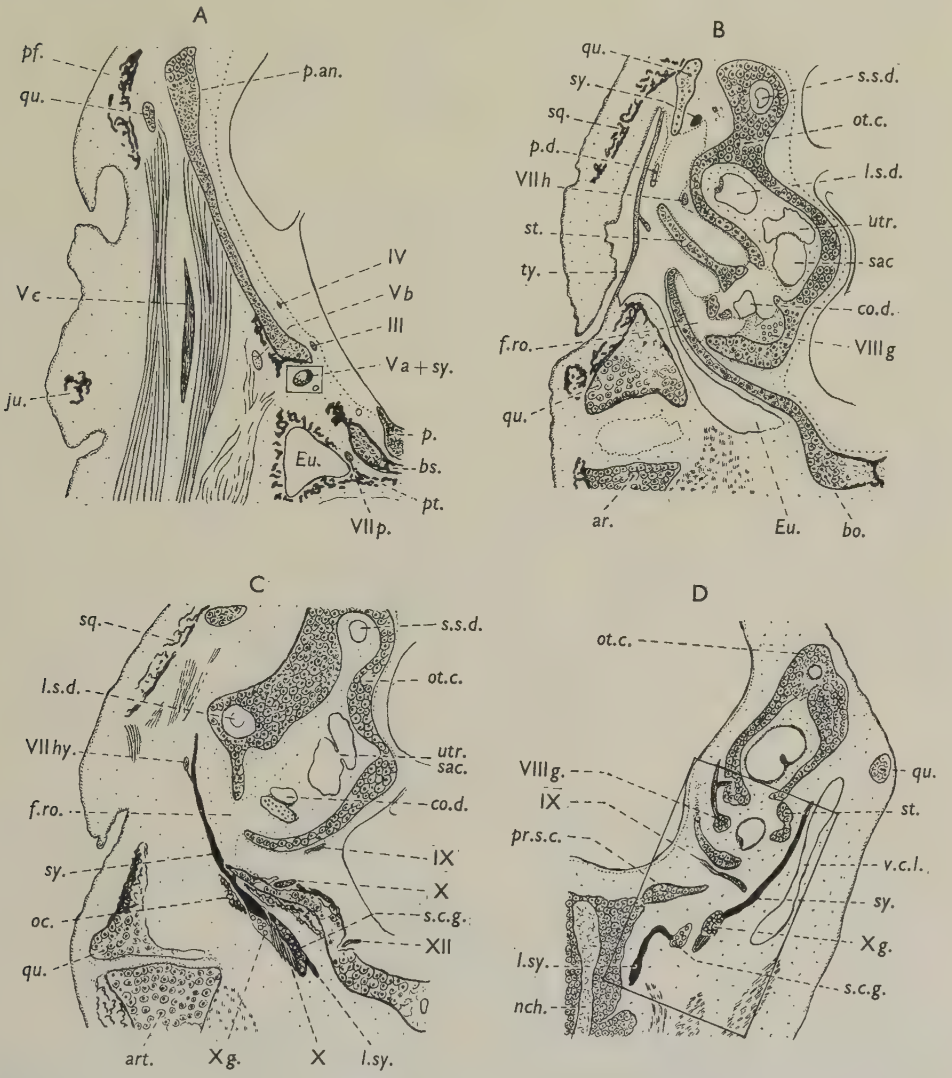
THE CRANIAL SYMPATHETIC SYSTEM AND INNERVATION OF THE NARIAL MUSCLES

The muscles are penetrated by terminal twigs of the lateral branch of the ethmoidal ramus of the ophthalmic nerve. Unstriped muscle in vertebrates always receives an autonomic (general visceral efferent) innervation. As the two components of the narial musculature work in a mutually antagonistic fashion they might be expected to receive a double nerve-supply from both the sympathetic and parasympathetic systems. In lizards and snakes, and also in birds, a motor ganglion which may be regarded as parasympathetic is situated on the course of the ophthalmic nerve in the anterior orbital region, usually in relation to the Harderian gland. This ganglion is known as the ethmoidal ganglion. In its immediate neighbourhood the ophthalmic nerve is joined by communicating branches made up of pre-ganglionic fibres from the palatine division of the facial nerve. We have been unable to find any comparable ganglion along the course of the ophthalmic nerve in Crocodilia, nor have we been able to demonstrate any communication between the ophthalmic and palatine nerves caudal to the region of the nostrils. The cranial parasympathetic ganglia in crocodiles consist of the ciliary ganglion on the course of the oculomotor nerve, the palatine ganglia on the course of the maxillary nerve where it communicates with the palatine nerve, and the mandibular (or submandibular) ganglion on the course of the chorda tympani in the lower jaw. An otic ganglion associated with the mandibular nerve and having roots from the glossopharyngeal nerve is not known in reptiles. Study of silver-stained material has failed to provide any evidence for the existence of post-ganglionic parasympathetic fibres originating in any of the ganglia described and passing to the narial muscles in branches of the ophthalmic nerve. It therefore seems probable that both the constrictor and dilator muscles are supplied entirely from the sympathetic system.

The sympathetic system of crocodiles shows certain remarkable features. There is a lateral sympathetic ganglionated trunk on each side of the neck, lying along the sides of the vertebral bodies, and a single median ganglionated trunk related to the ventral aspect of the spine. The cranial extremity of the median trunk forks into two branches which diverge to join the lateral trunks (see Shiino, 1914). Our own observations show that the median trunk is derived from the fusion of a second pair of trunks, originally paramedian in position, during later embryonic life. In early embryos these paramedian trunks and their ganglia are larger than the lateral trunks; later, however, the lateral sympathetic trunks and ganglia come to predominate.

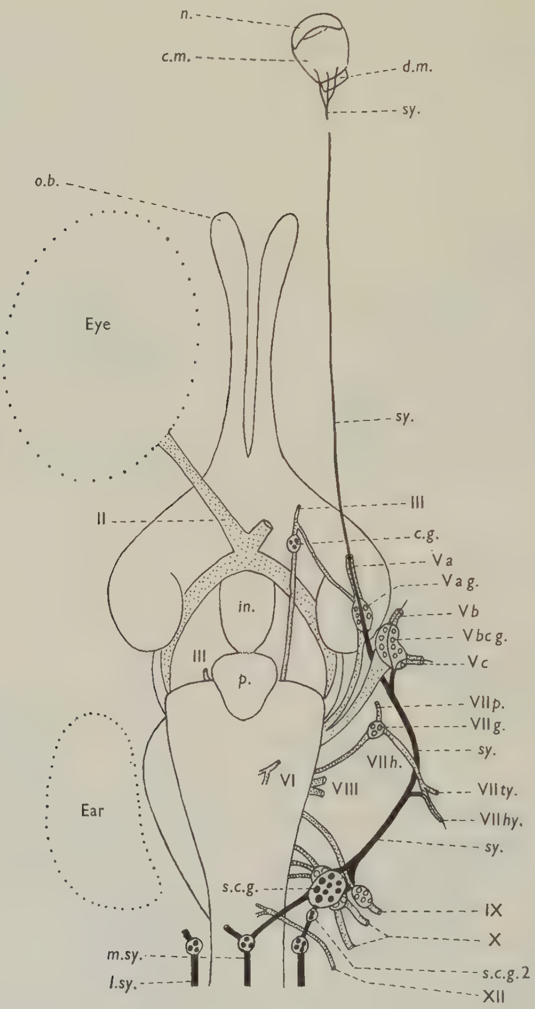
The most cranial of the series of sympathetic ganglia, which may be termed the superior cervical ganglion, is situated at the junction of the branch from the median trunk with the lateral trunk on either side (Text-fig. 5). During development a smaller group of ganglion cells may form in the root of the main ganglion derived from the lateral sympathetic trunk. This subsidiary ganglion was described by Shiino as the *ganglion cervicale secundum.* As embryonic growth proceeds the main ganglion (*primum*) becomes fused with the sensory ganglia of the glossopharyngeal and vagus nerves in the region of the jugular (metotic) foramen (Text-fig. 4C, D; Pl. 2, figs. 11, 12). Although the autonomic nerve cells are smaller than those associated with the sensory nerve fibres, the fusion between the autonomic and sensory ganglia becomes so intimate that the precise line of junction is usually obscured in non-selectively stained preparations of late embryonic material. In silver preparations, where the dendrites and axons are well impregnated, the extent and connexions of the sympathetic ganglion can be more readily determined.

The superior cervical ganglion gives off anteriorly the cranial sympathetic nerve (Text-fig. 5). In crocodiles the latter is a particularly stout bundle which reaches during late embryonic life a diameter greater than that of the posterior (hyomandibular) division of the facial nerve. After leaving the ganglion the cranial sympathetic nerve passes through a canal in that part of the occipital bone which ossifies ectochondrally beneath the basioccipital cartilage and processus subcapsularis, and which partly fills up the metotic fissure (Text-fig. 4C). The nerve then traverses the round window of the otic capsule (fenestra pseudorotunda: de Beer, 1937, p. 263) and crosses the caudal part of the secondary tympanic membrane which lies against the perilymphatic sac. It then passes medial to the hyomandibular division of the facial nerve to which it sends a communicating ramus. The latter is rather larger than the chorda tympani, which is small in crocodiles and arises from the hyomandibular branch just proximal to this point. The fibres of this communicating sympathetic branch appear to run distally with the hyoid ramus of the facial nerve, which is distributed to the sphincter colli muscle and to the muscles of the pinna-like organ which crocodiles possess. The significance of the presence of sympathetic fibres in the hyoid nerve is obscure.



Text-fig. 4. A–C, *Alligator*, 26 mm. head-length embryo. Transverse sections through side of head. A is at the level of the caudal part of the ophthalmic ganglion. The cranial sympathetic nerve, shown in black, has joined the root of V*a*. An enlarged view of the area within the rectangle is shown in Pl. 2, fig. 8. B is at the level of the stapes. The sympathetic nerve is running across the roof of the middle ear. C is at the level of the glossopharyngeal, vagal and superior cervical ganglia. The root of the superior cervical ganglion from the lateral cervical sympathetic trunk is shown. The cranial sympathetic nerve is giving off a branch to the hyoid ramus of VII. D. *Alligator*, 12 mm. *h-l.* embryo. Horizontal section through side of head showing the same structures as are figured in C, but at a rather earlier stage in development. The area inside the rectangle corresponds with that in Pl. 2, fig. 12. A–C × 10: D × 15.

The sympathetic nerve then runs obliquely across the crista parotica, a swelling of the otic capsule around the lateral semicircular duct, and medial to the dorsal process of the extra-stapes. It continues forwards above the stapes in the roof of the middle ear cavity, where it lies in a cleft between the otic capsule medially and the otic process of the quadrate laterally (Text-fig. 4B). It passes dorsal to the geniculate ganglion without apparently communicating with it. On reaching the prootic fenestra the sympathetic nerve crosses dorsally and then medially to the maxillary and mandibular portions of the trigeminal ganglion and gives off a small communi-



Text-fig. 5. Diagram showing sympathetic innervation of narial musculature in a crocodilian, seen from below. The muscles are shown intact. The cranial nerves are mostly figured on the right side of the diagram only, where the projecting part of the medulla is not shown. Sympathetic nerves in black, other nerve fibres in stipple. Autonomic ganglia shown by large black dots, sensory ganglia by circles. Approx. regions of orbit and inner ear shown by dotted lines.

cating branch to each. The nerve finally joins the root of the ophthalmic nerve and can be traced as a distinct bundle ventro-medially through the ophthalmicus ganglion (Text-fig. 4A; Pl. 2, figs. 8, 9).

It may be seen from Text-fig. 5 that whereas the superior cervical ganglion and its roots lie ventral to the vagus and hypoglossal nerves, the sympathetic nerve partly surrounds the glossopharyngeal root and runs dorsal to the facial nerve and the maxillo-mandibular root of the trigeminus.

After leaving the ophthalmic ganglion the fibres of the sympathetic nerve become scattered in the peripheral trunk of the ophthalmic nerve and those destined to supply the narial muscles enter the lateral ethmoidal ramus. In *Alligator* and *Caiman* this nerve runs forwards beneath the posterior edge of the roof of the nasal capsule for a short distance before leaving the capsule through the foramen epiphaniale. It then traverses the substance of the nasal gland and breaks up into several branches. In some species of *Crocodilus* at least, the lateral ethmoidal ramus passes dorsal to the nasal capsule on its way to the gland and does not become intra-capsular; consequently there is no foramen epiphaniale (see Bertau, 1935; de Beer, 1937, p. 266).

A number of stout nerve branches leave the front of the nasal gland and penetrate the narial musculature, mostly reaching it from its medial aspect (Text-fig. 1A). The nerve fibres in these branches to the muscles can be identified in silver preparations, ramifying between the muscle fibres and terminating in exceedingly fine, branching, beaded filaments on the muscle cells themselves (Pl. 2, fig. 10).

The nasal gland itself apparently receives a sympathetic supply from the ethmoidal nerves as they run among its acini; we were unable to trace any parasympathetic fibres to the gland from the palatine division of the facial nerve. It would therefore appear that the innervation of this gland, like that of the narial muscles, is derived entirely from the sympathetic system.

DISCUSSION

The Crocodilia have evolved an efficient mechanism for opening and closing the nostrils which is apparently unique among amniotes. The evolution of this mechanism, together with other special modifications of the anterior respiratory tract, may have been an important factor in the successful adaptation by the crocodiles to the ecological niche which they have occupied for so long a period in the earth's history.

A system of unstriped muscles, acting on the nostrils, has been described in certain salamanders by Bruner (1902). He believed that the muscles play an essential part in the respiration of these amphibians, which does not depend on movements of the ribs. Air is pumped into the respiratory tract by the action of the throat muscles, and intermittent closure of the nostrils is essential to prevent its escape from the nose. According to Bruner comparable muscles occur in frogs, but in these animals a new mechanism of narial closure involving movements of the striped masticatory muscles has also been developed. Bruner (1897) regarded the narial muscles of salamanders as homologous with those of crocodiles, but it is clear from his later (1902) account, that they follow a rather different pattern and that the constrictor muscle does not encircle the dilator.

Bruner (1902) suggested that the narial muscles of salamanders are partly responsible for the discharge of secretion from the nasal gland. It is possible that the narial muscles of crocodiles also play some small part in this process; movements of the muscles might bring about a slight compression of the anterior part of the gland or cause alterations in the lumen of its duct. Since only the anterior part of the duct runs through the circular muscle, however, and since the bulk of the gland lies well behind the narial muscles, it seems unlikely that the latter are of much importance in the control of secretion.

The disposition of the narial muscles of crocodiles, where an outer ring of circular fibres surrounds an inner bundle of longitudinal fibres, is not often paralleled among the unstriped muscle systems of vertebrates. A comparable arrangement is shown by the striped muscles in the tongues of lizards and snakes and the tongue-like rasping organs of lampreys, and enables these structures to be protracted and withdrawn. Among invertebrates the unstriped body musculature of annelids is segregated into an outer circular and an inner longitudinal coat, and alternate contraction of the two sets of muscles produces changes in the shape of the body which play an essential part in locomotion. Here, however, both circular and longitudinal muscles form tubes around the body cavity and do not form a solid mass like the crocodilian narial muscles.

The cranial sympathetic nerve in crocodiles was first observed by Fischer (1852) who described it in two parts as the *ramus recurrens ad nervum facialem* (from the maxillary nerve) and the *ramus communicans externus nervi facialis cum glossopharyngeo*. Shiino (1914) has subsequently given a more detailed account of it, but neither he nor Fischer correlated its large size with the presence of smooth muscles in the snout. In other reptiles the cranial sympathetic is represented by a fine nerve which brings the cervical trunk into communication with branches of the trigeminal nerve at points distal (from the brain) to its ganglia. No structure comparable with the superior cervical ganglion of crocodiles, birds and mammals is present, and the cranial sympathetic comes off the most anterior of the sympathetic ganglia which is situated at the root of the neck.

The cranial sympathetic nerve was examined in a newly hatched specimen of *Fringilla coelebs* (chaffinch) and was found to resemble the crocodilian condition except that it was distributed entirely to the maxillary and mandibular portions of the trigeminal ganglion. It did not communicate with the ophthalmic portion. The presence of a large motor root to the ophthalmic ganglion therefore appears to be a peculiarity of the Crocodilia, among amniotes at least.

In none of the Crocodilia examined were any motor ganglion cells found on the course of the cranial sympathetic nerve rostral to the superior cervical ganglion. Presumably, therefore, the nerve consists entirely of post-ganglionic fibres derived from cell bodies located in this ganglion or caudal to it. Preganglionic fibres may reach the ganglion either through the median or through the lateral cervical sympathetic trunks. Whether these alternative routes have any functional significance in relation to the antagonistic actions of the constrictor and dilator naris musculature remains a matter for conjecture.

SUMMARY

1. The narial musculature of Crocodilia consists essentially of a longitudinal band of fibres which draws back the posterior wall of the nostril and dilates the narial opening, and of a ring of circular fibres, contraction of which closes the nostril. The latter effect is apparently brought about indirectly as the result of the anterior part of the longitudinal muscle being compressed and forced against the back of the nostril. The latter is thus approximated to the anterior narial wall.

2. The narial muscles are unstriped in character and develop from mesenchyme *in situ*.

3. The muscles receive a sympathetic innervation from effector neurons whose cell bodies are probably located in the superior cervical ganglion and whose axons reach the snout via the cranial sympathetic nerve. The fibres of the latter join the lateral ethmoidal ramus of the ophthalmic division of the trigeminal nerve. The course of the cranial sympathetic nerve is described in detail.

4. The narial muscles of crocodiles are apparently unique among amniotes, and together with the other special modifications of the anterior respiratory tract, may have played an important part in the adaptation of these animals to their special ecological niche.

We are much indebted to the New York Zoological Society for a grant enabling us to purchase living crocodilians, to Dr Hugh B. Cott and Dr W. C. Osman Hill for gifts of material, and to Dr H. W. Lissmann of the Cambridge Zoological Department for allowing us to photograph a living crocodile in his charge. We are most grateful to Prof. J. D. Boyd for his helpful advice, and to Mr J. A. Fairfax Fozzard for taking the photographs.

REFERENCES

Bertau, M. (1935). Zur Entwicklungsgeschichte des Geruchsorgans der Krokodile. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **104**, 168–202.

Bruner, H. L. (1897). New nasal muscles in the Reptilia. *Anat. Anz.* **13**, 217–218.

Bruner, H. L. (1902). The smooth facial muscles of Anura and Salamandrina, a contribution to the anatomy and physiology of the respiratory mechanism of the amphibians. *Morph. Jb.* **29**, 317–364.

De Beer, G. R. (1937). *The Development of the Vertebrate Skull.* Oxford: The Clarendon Press.

Fischer, J. G. (1852). Die Gehirnnerven der Saurier. *Abh. Naturw. Hamburg*, **2**, 109–212.

Hoffmann, C. K. (1890). In H. G. Bronn's *Klassen und Ordnungen des Tier-Reichs*, vi. Leipzig: Winter.

Röse, C. (1893). Ueber die Nasendrüse und die Gaumendrüsen von *Crocodilus porosus*. *Anat. Anz.* **8**, 745–751.

Shiino, K. (1914). Studien zur Kenntnis des Wirbeltierkopfes. I. Das Chondrocranium von *Crocodilus* mit Berücksichtigung der Gehirnnerven und der Kopfgefässe. *Anat. Hefte*, **50**, 253–382.

EXPLANATION OF PLATES

Plate 1

Figs. 1, 2. *Crocodilus cataphractus*, living juvenile. Dorsal view of snout showing nostrils open in 1, partly closed in 2. ×2·5

Fig. 3. *Alligator mississippiensis*, embryo 12 mm. head-length. Horizontal section through snout showing right nostril and developing muscles. Haematoxylin and eosin. ×45.

Fig. 4. *Alligator*, embryo 26 mm. *h-l.* Transverse section through middle parts of narial muscles. Trichrome. ×85.

Fig. 5. *Caiman latirostris*, juvenile. Longitudinal section through epithelium of nostril showing dendritic pigment cells. Haematoxylin and eosin. ×400.

Fig. 6. *Caiman* juvenile. Longitudinal section through nostril. De Castro. ×25.

Fig. 7. *Alligator*, juvenile. Horizontal section through right nostril showing muscles. Plane of section corresponds with that in Text-fig. 2. Trichrome. ×25.

PLATE 2

Fig. 8. *Alligator mississippiensis*, embryo 26 mm. head-length. Transverse section through ophthalmic ganglion showing sympathetic root. Area shown corresponds with that in rectangle in Text-fig. 4A. Haematoxylin and eosin. ×220.

Fig. 9. *Caiman latirostris*, juvenile. Longitudinal section through ophthalmic ganglion showing sympathetic root. De Castro. ×85.

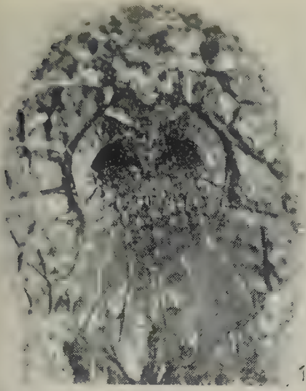
Fig. 10. *Caiman*, juvenile. High-power view of nerve ending on narial muscle fibre. De Castro. ×1400.

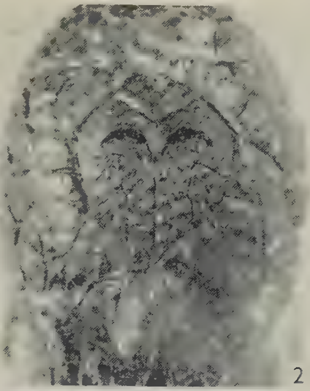
Fig. 11. *Caiman*, juvenile. Longitudinal section through superior cervical ganglion. De Castro. ×85.

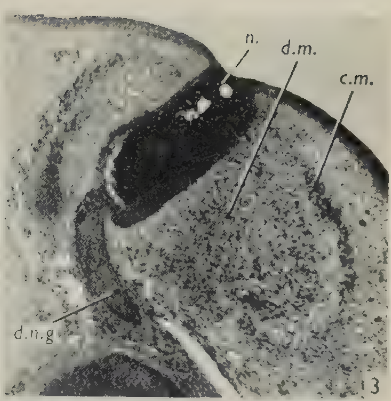
Fig. 12. *Alligator*, embryo 12 mm. *h-l.* Horizontal section through right side of head showing superior cervical ganglion, lateral cervical sympathetic trunk and cranial sympathetic nerve. Area shown corresponds with that in rectangle in Text-fig. 4D. Haematoxylin and eosin. ×45.

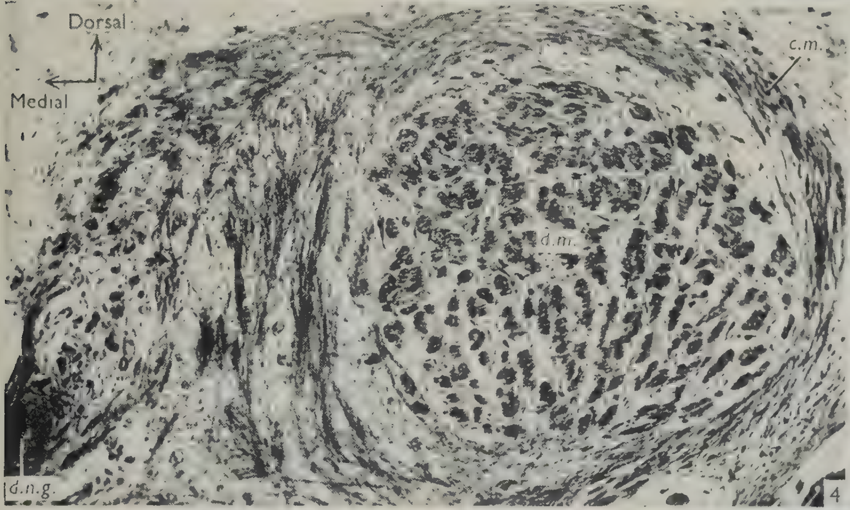
Abbreviations to text-figures and plates

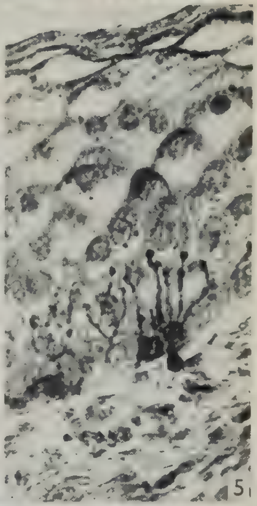
ar., *art.*	articular
bo.	basioccipital
bs.	basisphenoid
c.g.	ciliary ganglion
c.m.	constrictor muscle of nostril
c.n.sac.	cavity of nasal sac
co.d.	cochlear duct
c.t.	cavernous tissue
cup.	nasal cupola
d.m.	dilator muscle of nostril
d.n.g.	duct of nasal gland
Eu.	Eustachian tube
f.ro.	round window (fenestra pseudo-rotunda)
ju.	jugal
in.	infundibulum
l.s.d.	lateral semicircular duct
l.sy.	lateral cervical sympathetic trunk
m.sy.	median cervical sympathetic trunk
mx.	maxilla
mx.p.	palatine process of *mx*
n.	nostril
na.	nasal
nch.	notochord
n.ep	epithelium of nasal sac
n.g.	nasal gland
n.s.	nasal septum
n.sac.	nasal sac
o.b.	olfactory bulb
oc.	part of occipital bone ossifying ectochondrally
ot.c.	otic capsule
p.	pituitary
p.an.	pila antotica
p.d.	processus dorsalis of extra-stapes
pf.	postfrontal
pmx.	premaxilla
p.pmx.	palatine process of *pmx*
pr.s.c.	processus subcapsularis of basioccipital
pt.	pterygoid
qu.	quadrate
r.n.c.	roof of nasal capsule
sac.	saccule
s.c.g.	superior cervical ganglion
s.c.g. 2	ganglion cervicale secundum
sq.	squamosal
s.s.d.	superior semicircular duct
st.	stapes (columella auris)
sy.	cranial sympathetic nerve
t.	tooth
ty.	tympanic membrane
utr.	utricle
utr.-sac.	utriculo-saccular connection
v.c.l.	vena capitis lateralis
II	optic nerve
III	oculomotor nerve
IV	trochlear nerve
V*a*	ophthalmic nerve
V*a g.*	ganglion of V*a*
V*a l.*	lateral ethmoidal ramus of V*a*
V*a m.*	medial ethmoidal ramus of V*a*
V*b*	maxillary nerve
V*c*	mandibular nerve
V*bc g.*	maxillo-mandibular ganglion
VI	abducens nerve
VII*g.*	ganglion of facial nerve
VII*h.*	hyomandibular division of facial nerve
VII*hy.*	hyoid ramus of VII
VII*p.*	palatine division of facial nerve
VII*ty.*	chorda tympani
VIII	auditory nerve
VIII*g.*	ganglion of VIII
IX	glossopharyngeal nerve
IX*g.*	ganglion of IX
X	vagus nerve
X*g.*	ganglion of X
XII	hypoglossal nerve

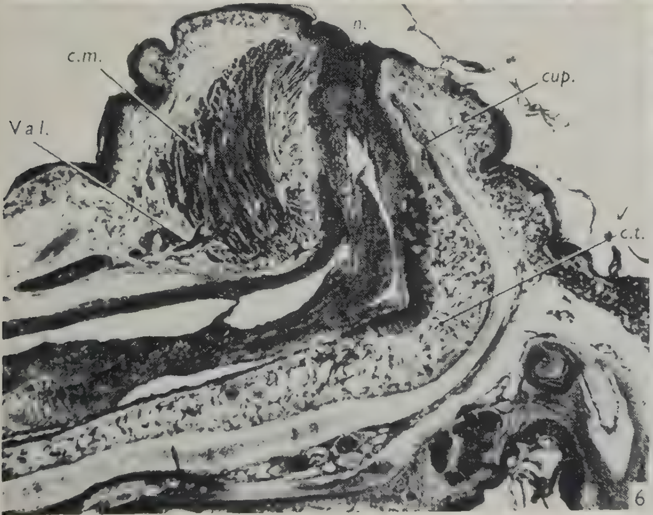


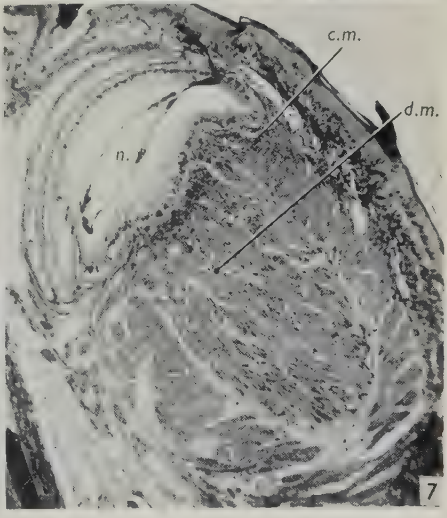




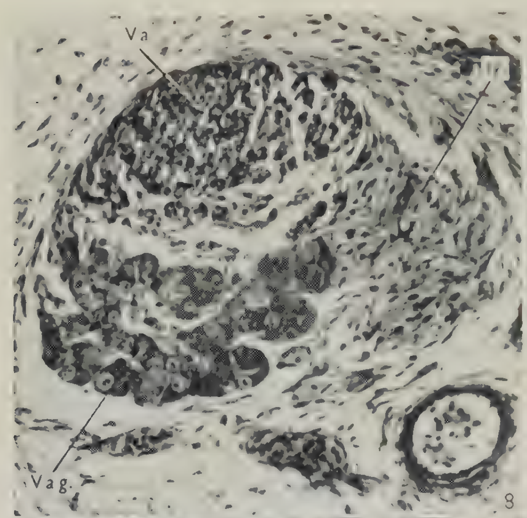








BELLAIRS AND SHUTE—NARIAL MUSCULATURE OF CROCODILIA



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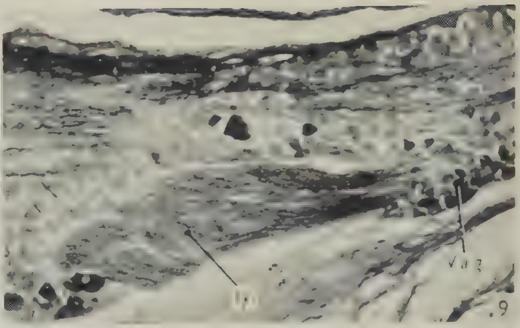

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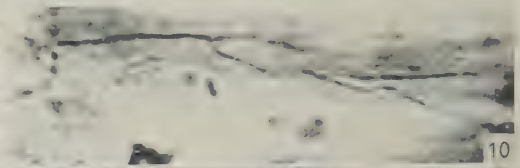
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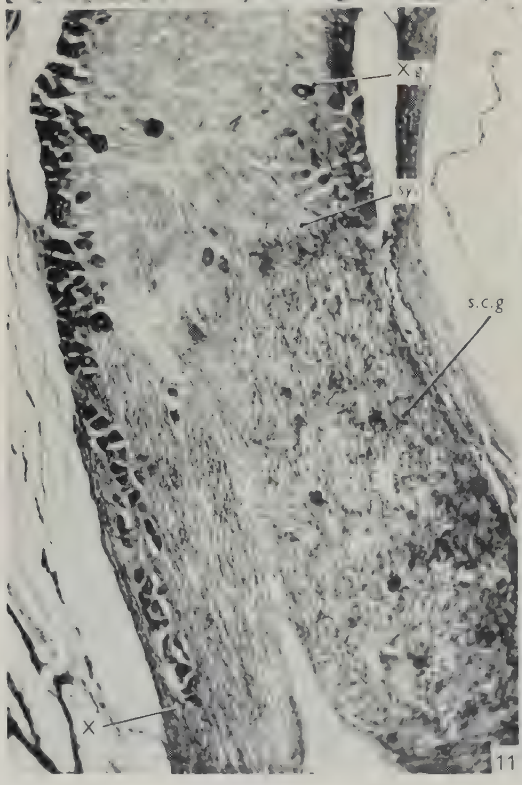

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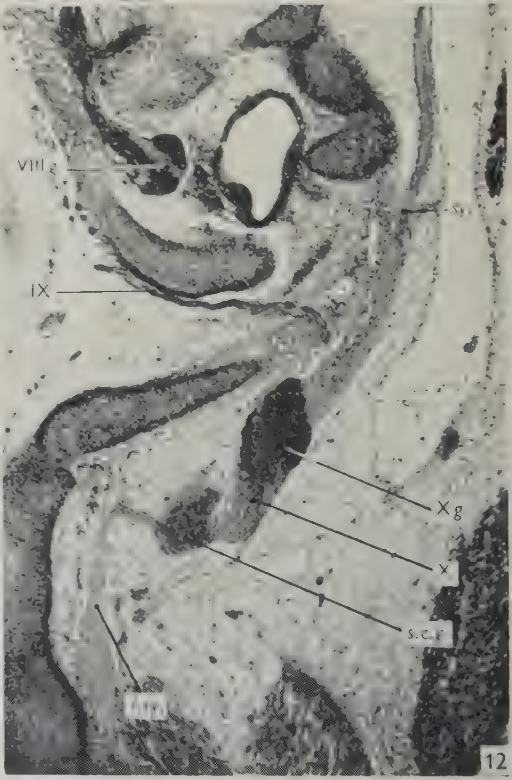

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BELLAIRS AND SHUTE—NARIAL MUSCULATURE OF CROCODILIA

THE VASCULARIZATION OF AUTOGRAFTS AND HOMOGRAFTS OF RABBIT SKIN

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This investigation was designed to answer the following questions:

(1) How soon are full thickness autografts of skin revascularized?

(2) Are homografts of skin revascularized as rapidly and as fully as autografts?

(3) If homografts are, in fact, revascularized in a manner similar to autografts, is subsequent failure of the circulation in homografts the immediate cause of their eventual destruction?

Previous investigations of these problems by various workers have given conflicting results. Medawar (1944), using stained sections of uninjected material, maintained that autografts and homografts of rabbit skin are revascularized at the same rate, by the ingrowth of capillaries from the underlying graft bed, and that revascularization is anatomically complete by about 4 days after grafting. He also described stagnation of the circulation and destruction of the blood vessels within the graft dermis at the time of homograft destruction.

On the other hand, Ham (1952) claimed, on the basis of Indian ink injected material, that homografts of skin, unlike autografts, are never revascularized. He attributed their eventual destruction to this failure of revascularization. He was supported in these views by Conway, Joslin, Rees & Stark (1952) who used a modified ear-chamber technique to study the circulation of grafts in living mice.

In view of these conflicting results, reinvestigation of the problem was undertaken, using intravenous injections of bromophenol blue as an indicator of the efficiency of the circulation within the grafts.

MATERIAL AND METHODS

Albino rabbits from a mixed stock maintained in the department were used. At each operating session the hair was clipped on the dorsum of the ears of a pair of rabbits, and five circular pieces of skin, 12 mm. in diameter, were cut from each ear. The full thickness of skin was removed, care being taken not to damage the subcutaneous vascular plexus. Of the twenty grafts cut at each session, sixteen were used as homografts and exchanged between the two rabbits. The remaining four grafts were replaced as autograft controls. The grafts were fixed in position by interrupted sutures of silk. No dressings were used. In all, eight pairs of rabbits were used. Of the total of 160 grafts, fewer than five failed to 'take' satisfactorily. The rabbits made no attempt to scratch the grafts and no protection proved necessary.

The absence of dressings allowed daily inspection of the grafts. At daily intervals following grafting, 4–6 ml. of 4 % bromophenol blue were injected over a period of about 1 min. into the marginal vein of the ear. The dye solution was prepared in the following way: 1 g. bromophenol blue was ground in a mortar with 15 ml. N/5-sodium hydroxide, and made up to 25 ml. with 0·9 % sodium chloride solution.

The properties of the dye which have made it valuable in this investigation are briefly as follows. Normally vascularized tissues are coloured intensely blue immediately on injection, and, conversely, tissues which are not normally vascularized are not immediately coloured. Dye will, however, enter non-vascularized tissues by a slow process of diffusion over a period of hours. Secondly, the dye is excreted fairly rapidly, and fully vascularized tissues are no longer coloured 24 hr. after the injection. Dye is removed from non-vascularized tissues far more slowly, however, and non-vascularized skin grafts are still blue 24 hr. after the injection. The colour changes seen in the normal skin of the ear serve, then, as a control for those occurring in the grafts. Finally, the dye is non-toxic, and animals tolerate repeated injections.

Serial biopsies of grafts were removed from seven of the sixteen animals studied. Each biopsy consisted of part or all of one homograft, including most of the underlying graft bed. The first biopsy was removed shortly before the anticipated time of homograft destruction, usually on the 7th day. Similar biopsies were then removed on each succeeding day, until homograft destruction was clearly evident to naked eye inspection.

The results obtained by bromophenol blue injection were checked, in a further pair of rabbits, by vascular injection of Indian ink. Each of these rabbits bore two homografts and one autograft on one ear. Both animals were sacrificed on the 7th day. At this time, the homografts on one animal were still healthy in appearance, while those on the second animal were clearly dying. After death the animals were perfused with warm physiological saline solution, and injected with filtered Indian ink by way of the common carotid artery. The ear was removed, fixed in 10 % formalin and cleared in xylol. Thick freehand sections of the grafts and adjacent normal skin were cut and mounted in D.P.X.

OBSERVATIONS

Before describing the changes occurring in grafted skin following dye injections, it may be of value to review the sequence of changes in the naked eye appearance of grafts in animals not receiving the dye injections. On the 1st day after grafting, the skin immediately surrounding the grafts is slightly reddened, as the result of a mild inflammatory reaction caused by the operative trauma. The grafts are pale and almost translucent. By the 3rd or 4th day, both autografts and homografts are bluish pink in colour, resembling the surrounding skin, which still shows slight signs of inflammation. By the 7th day, autografts and homografts are usually well healed, are pink in colour, and show a branny surface desquamation.

In most of the animals of this series, homograft destruction had begun by the 8th day; the longest period of survival noted was 15 days. The gross signs of homograft destruction are quite characteristic. Dying homografts are thicker than the autograft controls, and raised above the general skin surface. They are discoloured—sometimes a dusky red colour, sometimes a dark grey, as if the surface of the graft had been shaded by a soft lead pencil. These colour changes are noted simultaneously in all the homografts borne by any particular animal. During the course of 3 or

4 days after the onset of destruction, the homografts become dry and dark in colour, and the superficial parts of the grafts are finally shed as hard black scabs.

In animals injected with dye, there is an immediate blue coloration of the normal skin, evident even before the injection is completed. The colour is commonly more intense in the general body skin than in that of the ears, the difference being more marked when the ears are cold. Colour changes produced in the grafts by the injection depend upon the extent to which revascularization has occurred and, therefore, upon the time which has elapsed since the placing of the grafts.

The following description summarizes the results obtained by dye injection on the morning of each of the first 5–6 days after grafting.

Day 1 (1st day after grafting)

Immediately after injection, the grafts showed no trace of the dye, and were pale, in striking contrast to the blue-coloured normal skin. After injection, the grafts gradually acquired a blue colour, but even after 4 hr. they remained a paler blue than the normal skin. It is clear that dye enters the non-vascularized grafts slowly by diffusion from the surrounding tissues.

Day 2

On the morning of the 2nd day, the normal ear skin was completely free of the dye injected on day 1. The grafts, on the other hand, remained a pale blue colour. The removal of dye by diffusion alone is, like its entry, evidently a slow process.

The animals now received a second injection of dye, which confirmed the fact that the grafts were not yet revascularized. The injection produced no immediate deepening of the pale blue colour of the grafts. Some 5 hr. after injection the grafts were as blue as the surrounding normal skin.

Day 3

On the morning of the 3rd day, the dye injected 24 hr. previously had disappeared from normal skin. Traces of dye remained in both autografts and homografts in all except one of the animals.

A further injection of the dye in most cases failed to produce immediate coloration of the grafts. In one animal—that from which all traces of the previous day's injection had disappeared—both autografts and homografts became coloured blue as quickly and as intensely as the surrounding skin. In this case, then, revascularization was already complete within 72 hr. after grafting. This represents the most rapid attainment of full revascularization in this series. In the remaining cases it was noted that the grafts attained the same colour as the surrounding skin much more rapidly than on days 1 and 2. Usually within an hour of injection, no difference could be noted between the colour of the grafts and that of normal skin.

Day 4

Most of the animals showed no retention in the grafts of the dye injected on day 3. A further injection produced immediate and complete coloration of both autografts and homografts. No differences were observed in either the rate or intensity of coloration of autografts and homografts.

Day 5

In two of the animals, autografts and homografts showed retention of the dye injected on day 4. Of these two, complete injection was obtained in one later in the day, and in the other, on the morning of the 6th day.

In all the other animals no traces of dye were seen in the grafts on the morning of the 5th day, and a further injection merely confirmed the fact that vascularization was now complete.

Homograft destruction

The time relationship between the onset of circulatory failure and the appearance of histological evidence of epidermal destruction may be illustrated by detailed reference to three of the seven animals which received further dye injections after the time of revascularization and from which serial biopsies were removed and studied.

Rabbit no. 191

Dye injections showed all the grafts to be fully vascularized by the 4th day after grafting. Biopsies were taken of half or all of one homograft on each of the following days: 5, 7, 8, 9, 10, 11, 12, 14, 15 and 16.

The first evidence of circulatory failure was noted on the 14th day, when the remaining homografts were slightly purplish in colour as compared with the healthy pink autografts. Dye injection produced immediate and full coloration of the autografts, but only patchy coloration of the homografts. A biopsy taken at this time shows the homograft epidermis to be histologically normal (Pl. 1, fig. 1).

Injection on the following day (day 15) again produced delayed and patchy coloration of the homografts. Histological signs of beginning epidermal destruction are now evident: the infiltration of the epidermis by mononuclear leucocytes, and the appearance in the epidermis of small irregular clear spaces (Pl. 1, fig. 2). The biopsy removed on the following day (day 16) shows advanced destruction of the epidermis. The cytoplasm of the epidermal cells is now eosinophilic, and the nuclei are pyknotic. In isolated patches, the epidermis is separated from the dermis by mononuclear cells, many of whose nuclei are fragmented (Pl. 1, fig. 3).

Rabbit no. 170

Revascularization was complete on the 4th day after grafting. Biopsies were removed on days 6, 7, 8 and 9. Injection on day 7 produced immediate coloration of the autografts and homografts, and the homograft epidermis was histologically normal (Pl. 1, fig. 4). On the following morning (day 8), the homografts were reddish purple in colour and injected dye failed to penetrate them. Two homograft biopsies were removed, and both showed clear signs of epidermal destruction—nuclear fragmentation, epidermal separation and infiltration of inflammatory cells (Pl. 1, fig. 5).

Rabbit no. 185

Autografts and homografts were fully revascularized by the 5th day after grafting. On the 6th day, the grafts were clear of dye injected on the previous day, and no dye injection was given. On the 7th day, the autografts were pink and healthy in appearance but the homografts were a grey colour. Injected dye failed to penetrate the homografts immediately. Dye did, however, enter the grafts slowly by diffusion. The epidermis was histologically normal.

On the following morning (day 8), the homografts were dark grey in colour. Injected dye failed to penetrate the homografts and two homograft biopsies showed advanced epidermal destruction.

These three cases provide a useful summary of results obtained in the whole series, since they illustrate the two extremes and the mean of the varying time relationship between vascular breakdown and epidermal destruction. Vascular breakdown preceded epidermal death by 24 hr. in rabbit 185 and by 48 hr. in rabbit 191; in rabbit 170 the two events occurred simultaneously. In three of the four other animals from which serial biopsies were removed, vascular destruction preceded histological signs of the epidermal death by 24 hr. and in the fourth the two events occurred at the same time.

Indian ink injection

Sections of the grafts from the animal which was sacrificed and injected with Indian ink prior to the onset of homograft destruction show clearly the presence of injected vessels in the dermis of both autograft and homograft (Pl. 2, fig. 6). This material provides conclusive proof that skin homografts are revascularized.

In the second animal homograft destruction had already begun. It was injected before death with bromophenol blue. The single autograft was immediately coloured blue, but dye failed to enter the homografts. The animal was then killed and injected with Indian ink. In the cleared thick sections blue dye is readily seen. It is present in the normal skin of the inner surface of the ear, in the cartilage of the ear, in the graft bed and in the deep one-third or so of the graft dermis. The abundant vessels of the graft bed are fully injected with Indian ink, but ink is completely absent from the vessels of the graft dermis (Pl. 2, fig. 7). The vessels of the graft dermis are clearly seen, however, since they are packed with masses of red blood corpuscles. Some haemorrhages around the vessels are also seen. The vessels in the dermis of the control autograft are filled with ink (Pl. 2, fig. 8). It is evident that Indian ink does not enter the vessels of the homograft when vascular breakdown has begun, and that dye enters the homograft slowly, and independently of the blood vessels, by diffusion.

DISCUSSION

The use of highly diffusible non-toxic vital dyes as an indicator of the relative vascularity of different tissues was first extensively used by Rous & Gilding (1929). They established the fact that, in rabbits which had been bled, 'to all intents and purposes...the circulation had ceased to be effective in the skin areas which bromophenol blue...failed to penetrate'. In an earlier study Rous (1926) used phenol red as an indicator of the pH of skin autografts in mice. He was primarily concerned with the acidity of autografts prior to vascularization, but it is of interest to recall that he noted incidentally that 'by the end of a week the "take" is usually perfect and the phthalein coloration differs practically not at all from that of the host'. He correlates the immediate entry of the dye and the loss of acid reaction in the graft with the restoration of full vascularity. The reason for the longer time required for full vascularization in Rous's material as compared with that determined in the present study is not far to seek: his grafts were thicker, comprising 'the full thickness of the integument and...in addition...a varying quantity of fat and the thin layer of striped muscle' (panniculus carnosus). Clearly the time required for full vascularization will depend on the thickness of the graft which the new vessels have to penetrate. That many of Rous's grafts survived after a period of relative ischaemia of as long as 7 days serves merely to emphasize the resistance of grafted skin to adverse conditions.

In the present experiments the normal ear skin serves in two ways as a control in the interpretation of the colour reaction of grafted skin. Normal skin is coloured intensely immediately on injection. So long, therefore, as grafts fail to become immediately coloured, it can be concluded that they are not fully revascularized. Moreover, normal skin is completely cleared of dye within 24 hr. of injection. Grafts which retain dye after this interval are not yet fully revascularized.

Repeated daily injections of dye can, therefore, yield accurate information as to the rate of revascularization of homografts and autografts, and the main conclusions emerging from the observations presented above are these:

(1) There are no differences in the rate or extent of revascularization of homografts and autografts in any single animal.

(2) Full revascularization is in most cases achieved between 4 and 5 days after grafting. In two animals, full injection was obtained as early as 72 hr. after grafting. In one case, revascularization did not appear to be complete until the 6th day.

The method also gives accurate information about the time of secondary vascular breakdown and cessation of active circulation in homografts in relation to the onset of epithelial destruction as revealed by histological study of biopsies. Epithelial destruction is usually far advanced within 24 hr. of failure of the circulation of homografts. During this time fluid enters the grafts by a slow process of diffusion, just as it did during the initial 3 days or so following grafting. The fact that epithelial breakdown follows so rapidly upon vascular breakdown argues strongly against the idea that the one is due to the other. Since the epithelium survives for periods of 3 days or more prior to initial revascularization of the graft dermis, it could be expected to survive secondary vascular breakdown by as long a period. That it does not do so indicates that some factor other than ischaemia is responsible for its destruction.

The findings of this investigation finally and certainly exclude the possibility of non-specific vascular factors playing any significant role in homograft destruction.

Since dye—and therefore tissue fluid—continues to enter homografts by a slow process of diffusion following secondary vascular breakdown, the fact that homografts fairly rapidly become dry and hard demands some explanation. It seems likely that the death of the epidermis allows a more rapid loss of fluid from the surface of the graft than can be made good by diffusion from below.

Some attempt must be made to explain the discrepancy between our findings and those reported by Ham (1952) and by Conway, Joslin & Stark (1951), Conway, Stark & Joslin (1951) and Conway, Joslin, Rees & Stark (1952). Ham's (1952) observations were apparently restricted to a single animal (pig), which received a succession of full thickness autografts and homografts on different days. The animal was killed when the first grafts had been in position for 10 days, and was injected with Indian ink. The 7-day autograft was partially injected, and full injection of the 10 day autograft was obtained. There was, however, no revascularization of either the 7- or the 10-day homograft. The failure to obtain any injection at 10 days may simply mean that at this time the homograft and its blood vessels were already undergoing destruction. This interpretation is supported by the results obtained from Indian ink injected in the present investigation.

Conway, Joslin & Stark (1951) reported an ingenious modification of the ear chamber technique, and this was used by Conway, Stark & Joslin (1951) to study in the living animal the vascularization of autografts, and by Conway, Joslin, Rees & Stark (1952) to study homografts. The method is not, however, entirely suited to the problem, since on the authors' own admission, the grafts are so opaque that it is difficult to be certain about the penetration of vessels into the grafts from the underlying graft bed. Conway *et al.* (loc. cit.) concentrated their attention, therefore, on host vessels growing inwards towards the periphery of the graft. They maintain (1952) that vessels approaching the periphery of homografts at 7–8 days fail to enter the graft, and on this observation they base their claim that homografts never become vascularized. Our interpretation of their findings is briefly this: that autografts and homografts had, by about 5 days, become revascularized from below; that this revascularization was not clearly evident by the method used; and that the failure of vessels to enter the periphery of the homografts at 7–8 days indicated that homograft destruction had already begun.

SUMMARY

1. The rate and extent of revascularization of full thickness fitted autografts and homografts of rabbit ear skin have been studied by intravenous injections of a diffusible vital dye, bromophenol blue.

2. In any particular animal of the sixteen studied, no difference could be observed in the rate or extent of revascularization of autografts and homografts. In the majority of cases revascularization was completed between the 4th and 5th days after placing the grafts.

3. Illustrations of Indian ink injected material are presented which provide conclusive visual proof that homografts, like autografts, are revascularized.

4. Breakdown of the blood vessels of the dermis is part of the general pattern of homograft destruction. Epidermal destruction is usually well advanced within 24 hr of vascular breakdown. Since this time interval is so short, it is concluded that the vascular breakdown is not the immediate cause of the death of homograft epidermis.

Our thanks are extended to Prof. G. M. Wyburn for criticism of the manuscript, and to Mr G. Marshall and Mr C. Vernon for technical assistance. Part of the expense of this investigation was defrayed by a grant from the Medical Research Council, which is gratefully acknowledged.

REFERENCES

Conway, H., Joslin, D. & Stark, R. B. (1951). Observations on the development of circulation in skin grafts. I. Technique of adaption of transparent chamber to study of circulation in skin grafts. *Plast. reconstr. Surg.* **8**, 194–203.

Conway, H., Stark, R. B. & Joslin, D. (1951). Observations on the development of circulation in skin grafts. II. The physiologic pattern of early circulation in autografts. *Plast. reconstr. Surg.* **8**, 312–319.

Conway, H., Joslin, D., Rees, T. D. & Stark, R. B. (1952). Observations on the development of circulation in skin grafts. III. Morphologic changes observed in homologous skin grafts. *Plast. reconstr. Surg.* **9**, 557–564.

Ham, A. W. (1952). Some histophysiological problems peculiar to calcified tissues. *J. Bone Jt. Surg.* **34**, A, 701–728.

Medawar, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176–199.

Rous, P. (1926). The relative reaction within living mammalian tissues. VI. Factors determining the reaction of skin grafts. *J. exp. Med.* **44**, 815–834.

Rous, P. & Gilding, H. P. (1929). Studies of tissue maintenance. I. The changes with diminished blood bulk. *J. exp. Med.* **50**, 189–211.

EXPLANATION OF PLATES

Plate 1

In each of the three succeeding figures, the graft is uppermost, and the non-vascular ear cartilage appears as a pale horizontal band. Below the cartilage is the normal skin of the inner surface of the ear.

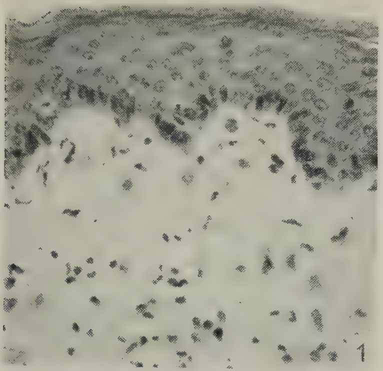
Fig. 1. Rabbit 191. Biopsy of homograft, day 14. Haematoxylin and eosin. × 225.
Fig. 2. Rabbit 191. Biopsy of homograft, day 15. Haematoxylin and eosin. × 225.
Fig. 3. Rabbit 191. Biopsy of homograft, day 16. Haematoxylin and eosin. × 225.
Fig. 4. Rabbit 170. Biopsy of homograft, day 7. Haematoxylin and eosin. × 225.
Fig. 5. Rabbit 170. Biopsy of homograft, day 8. Haematoxylin and eosin. × 225.

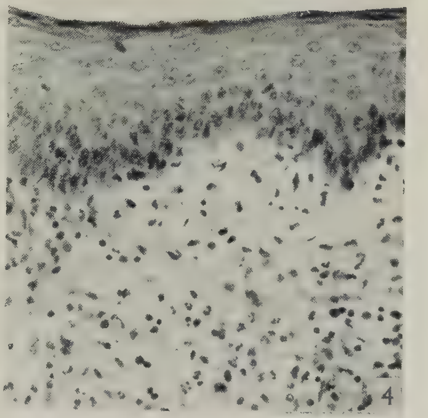
Plate 2

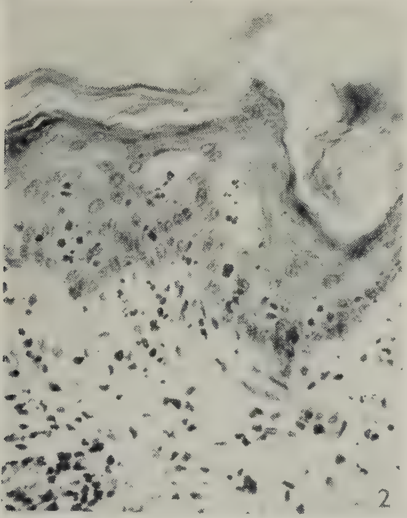
Fig. 6. Thick, cleared section of skin homograft from rabbit sacrificed and injected with Indian ink before onset of destruction of homograft. × 80 approximately.

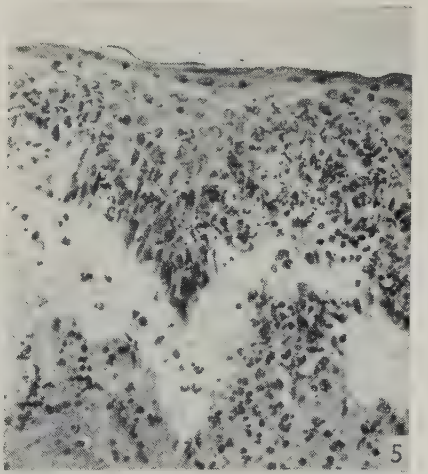
Fig. 7. Thick, cleared section of skin homograft from rabbit sacrificed and injected with Indian ink after time of onset of homograft destruction. × 80 approximately.

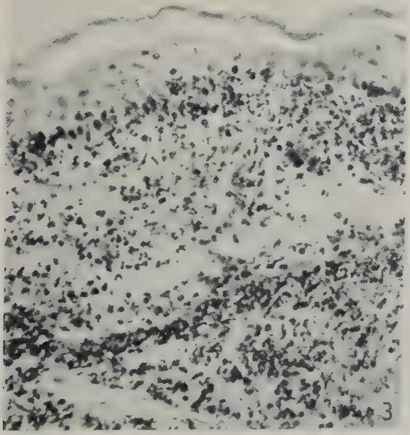
Fig. 8. Thick, cleared section of skin autograft from same rabbit as fig. 7. × 80 approximately.



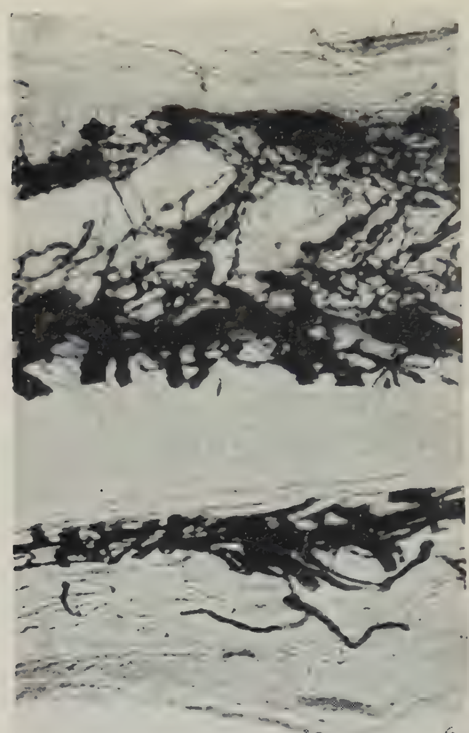








SCOTHORNE AND McGREGOR—VASCULARIZATION OF AUTOGRAFTS AND HOMOGRAFTS OF RABBIT SKIN



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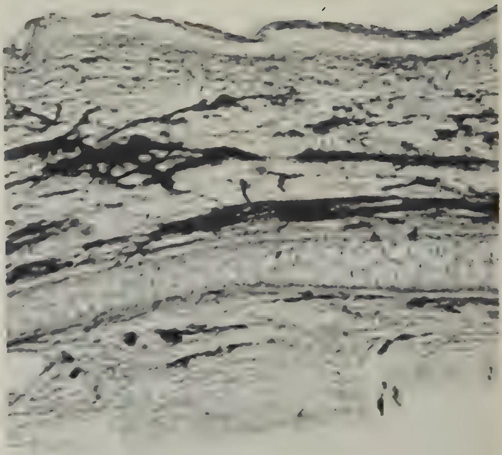

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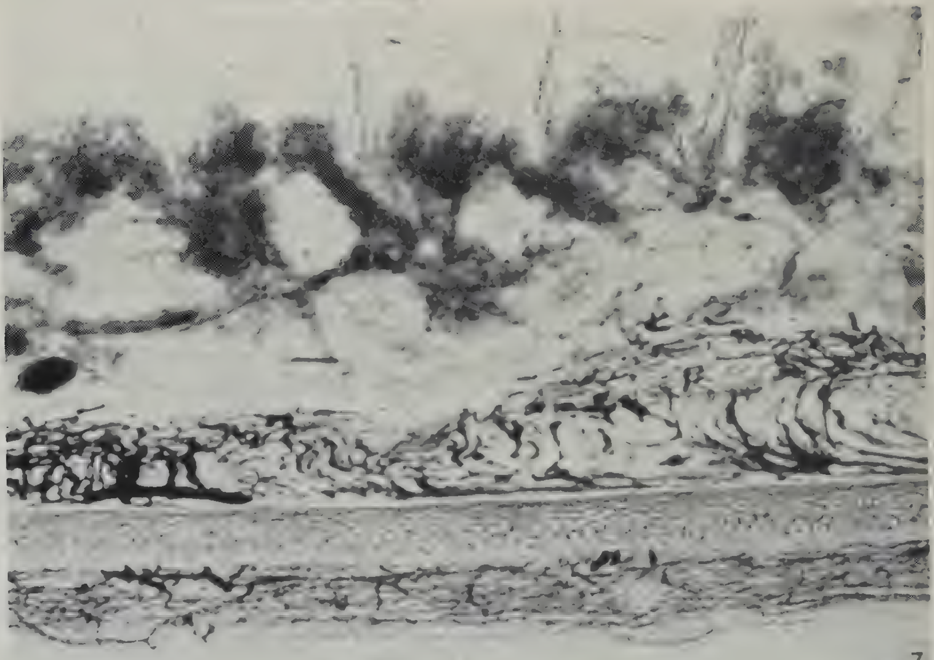

7

SCOTHORNE AND McGREGOR—Vascularization of autografts and homografts of rabbit skin

DENDRITIC ORGANIZATION IN THE NEURONS OF THE VISUAL AND MOTOR CORTICES OF THE CAT

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INTRODUCTION

The manner of transmission of the nerve impulse from one neuron to another in the cerebral cortex is unknown. Few terminal buttons are to be seen on the cell bodies of cortical neurons, and the nature of the synapsis is obscure. It is possible to approach the problem of cortical organization in another way. In addition to studying the detailed appearance of particular synaptic junctions, one may attempt to assess the general principles of neuronal interaction from a consideration of the geometrical relationships subsisting between the neurons and between the neurons and the incoming axons. This could be done by measuring: (1) the density of packing of the cells; (2) the pattern of branching of the dendrites as shown by their diameter, number and length, and direction; (3) the pattern of branching of the axons. The present paper describes the results of studies on the dendritic branching pattern in the visual and motor cortices of the cat and the density of packing of the cells in the visual cortex. The axonal branching pattern will be discussed in a subsequent paper.

The dendritic organization of the cerebral cortex has been little studied in recent years. Ramón y Cajal (1894, reproduced 1911) pointed out changes occurring during ontogeny and compared them with differences found in the phylogenetic series. The investigations of Lorente de Nó (1922, 1933, 1934) and of O'Leary (1941) on cortical histology are of outstanding importance, but, apart from a short paper by Bok (1936), no other recent detailed studies of dendrites seem to have been made.

METHODS

It has been found possible consistently to stain cortical neurons with the Golgi-Cox method in the following way:

(1) Cats are anaesthetized with nembutal (ether leads to excessive bleeding) and pieces of cortex not more than 4 mm. thick are removed from the living animal. These are rapidly rinsed in warm saline and put into about 50 ml. of the staining solution in a small stoppered bottle, the tissue resting on a piece of cotton-wool.

Staining solution—to be freshly prepared

5% aqueous potassium dichromate	20 ml.
5% aqueous mercuric chloride	20 ml.
Distilled water	40 ml.

Mix together and then, with continuous stirring, add

5% aqueous potassium chromate	8 ml.

(2) The bottles are left undisturbed in the dark at room temperature for 8 weeks (a shorter time for kittens and rabbits).

(3) The material, without previous washing, is first rinsed in a mixture of equal parts acetone and absolute alcohol, and then dehydrated in this mixture in a stoppered bottle for 24 hr. at 37° C.

(4) The material is transferred to a mixture of equal parts ether and absolute alcohol for 4 hr., and then,

(5) Placed in 5% collodion for 24 hr. and then in 12% collodion for 12 hr.

(6) Mounted on a fibre block in 12% collodion and hardened in chloroform vapour.

(7) Sections 100–200 μ are cut in 70% alcohol on a sliding microtome.

(8) The sections are transferred to distilled water to which two to three drops of a wetting agent have been added.

(9) Reduction is made in a fairly large volume of 5% aqueous potassium sulphite to which a few drops of 5% aqueous oxalic acid have been added. (The addition of oxalic acid clears the background, leaving it colourless. I owe this suggestion to Mr G. Hyde.)

(10) The sections are rinsed in distilled water for 10 min., transferred to a mixture of equal parts absolute alcohol and chloroform, and then put on the slide, flattened with filter-paper and coated with 1% collodion.

(11) The slide is transferred to a mixture of equal parts absolute alcohol and chloroform.

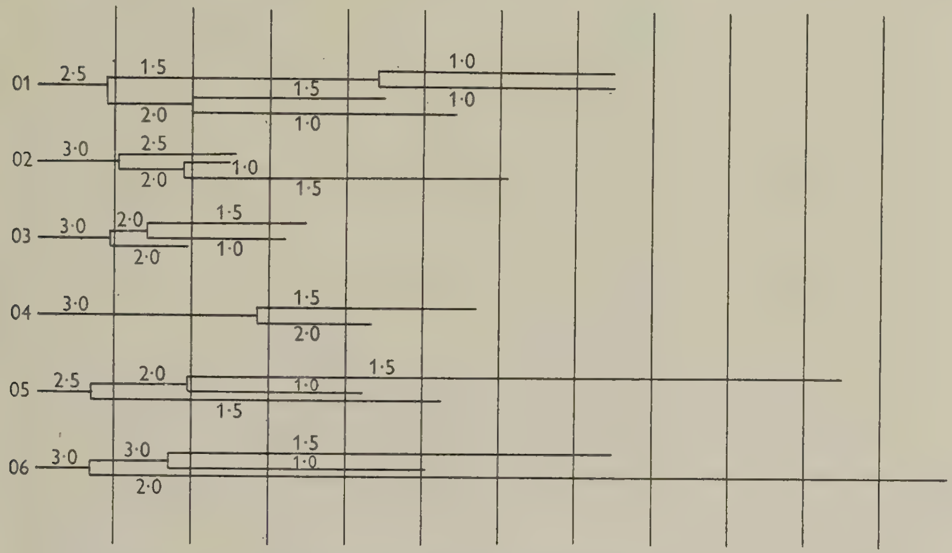
(12) The preparation is cleared in terpineol, mounted in dammar xylol and transferred to the incubator to dry. It may be necessary to add more balsam during the drying process. No satisfactory plastic mountant has been found, and all suggested methods for applying cover-slips have led to rapid fading.

Measurements

The measurements were made using a binocular microscope and a 4 mm. objective with graticules in the eyepieces. The graticules were calibrated against a stage micrometer. Since it is impossible to use an oil-immersion objective with thick sections, distances less than 2 μ are difficult to estimate; consequently there may be a rather large error in the measurements made on small diameters. Two corrections to the measured lengths are necessary when the dendrites do not run parallel to the plane of section since, first, the observed length is the projection of the true length on the plane of section and, secondly, all measured depths are of apparent depths due to the refractive index of the mountant.

In order to catalogue the dendrites quickly and in such a way that the relations of any particular branch could be found easily, the following convention was adopted. Branches arising directly from the perikaryon were numbered 01, 02, 03, etc. The next branches have numbers 011, 012, 021, 022 where 011 and 012 are the two branches formed from 01. Very occasionally, when a branch gave rise to three new branches, there would be a branch having a number of the form 013.

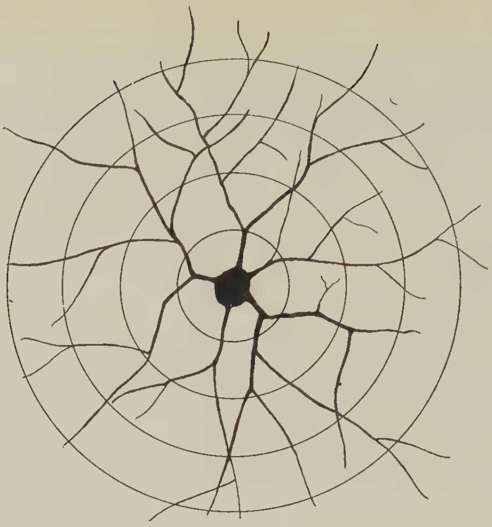
Subsequently, measurements of length and diameter were plotted on diagrams of the kind shown in Text-fig. 1, each cell being allotted a separate sheet. In these diagrams the directions of the branches are ignored but the lengths are plotted to scale. The lengths of the short vertical lines have no meaning, for these lines merely indicate the position of branch points. Long vertical lines are drawn across the diagram at distances representing 20μ. The figure alongside any branch is the mean value of its diameter; where branches are long and have a high taper, two such diameters are noted. Short thick perpendicular lines at the end of a branch show that the branch has been cut at this point. From diagrams of this kind it is easy to find the numbers of dendritic branches and terminations at different distances from the perikaryon.



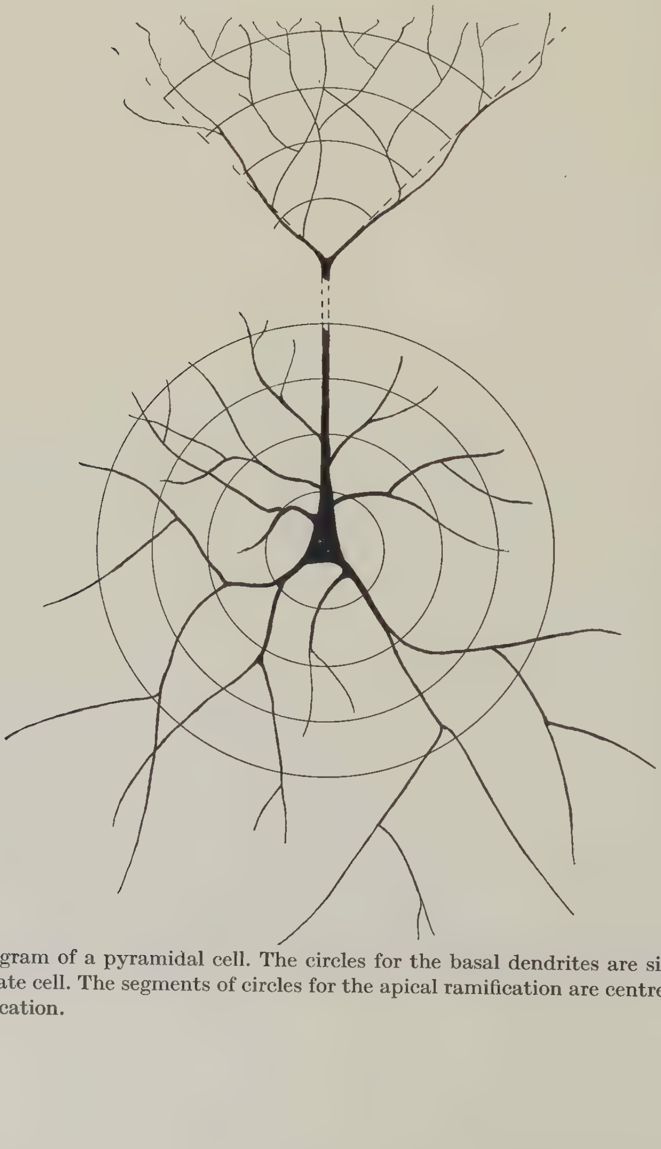
Text-fig. 1. An example of the method of graphical representation of the lengths of dendritic branches for a single neuron.

Sampling

The sampling of the cells to be measured is difficult. The cells suitable for measurement in any section have already been partly selected in an uncontrollable manner. The staining method itself is selective and even with the greatest care it is impossible to ensure perfect orientation of the knife. Moreover, even under the best conditions, there will be an excess of complete small cells in any section. However, since all the types described by other workers have been seen, there are grounds for believing that cells of all shapes and sizes have been stained; consequently, there is no reason to suppose that bias has been introduced. The number of complete large cells in any one section is small and the rule was adopted that, having measured a number of small cells at various cortical depths, all the large complete cells in any section were measured. Cells have been classified into two broad categories, stellate and pyramidal; the former are those with a spherical or ellipsoidal perikaryon and with dendrites symmetrically disposed around the perikaryon, while the latter have a pyramidal perikaryon with a definite apical dendrite. Photographs of these two types are shown in Pl. 1 and Text-figs. 2 and 3 show diagrams of the same types. Only six of the cells could not be classified on this basis and three of these were of the inverted pyramid type. These non-classified cells have not been further considered in this paper.



Text-fig. 2. Diagram of a stellate cell showing a series of circles with increasing radii centred in its perikaryon



Text-fig. 3. Diagram of a pyramidal cell. The circles for the basal dendrites are similar to those for the stellate cell. The segments of circles for the apical ramification are centred on the main apical bifurcation.

Table 1. *General data relating to neurons from the visual and motor cortices of the cat*

Cell no. (1)	Type (2)	Depth (μ) (3)	Thickness of cortex (μ) (4)	Volume of perikaryon (μ^3) (5)	Surface of perikaryon (μ^2) (6)	Total length of dendrites (μ) (7)	No. of dendritic branches (8)	Tangential extent of dendrites (μ) (9)	No. of proximal branches (10)
					Visual area				
6	P	420	1,850	1,680	660	2,534	49	365	4
8	S	865	1,850	4,850	1,390	3,405	71	350	10
9	P	1,260	1,700	4,080	1,270	4,962	79	265	10
10	P	840	1,700	4,310	1,240	3,970	78	340	6
11	P	1,470	1,700	19,620	3,870	4,832	78	340	9
12	P	1,050	1,700	9,160	2,250	2,848	49	200	4
13	P	250	1,700	1,030	470	1,736	46	250	5
15	S	1,050	1,700	1,700	690	1,129	26	250	4
20	S	840	1,900	9,010	1,700	1,529	30	225	7
21	P	950	1,900	3,560	1,110	2,521	47	250	4
22	S	685	1,900	3,890	1,390	1,523	34	160	6
23	S	714	2,000	14,140	2,830	3,775	38	480	7
					Motor area				
A	P	1,080	1,440	11,550	2,520	6,384	75	565	6
B	P	364	1,440	700	390	2,468	44	225	5
C	P	396	1,440	1,060	510	2,205	40	220	7
D	P	1,220	1,440	540	310	1,596	41	200	5
E	S	893	1,440	1,770	710	4,895	62	370	5
F	S	473	1,440	5,580	1,520	2,096	28	420	5
G	P	392	1,440	680	360	2,377	38	260	6
H	S	730	1,440	8,180	1,960	3,043	36	525	8
I	P	690	1,440	1,280	570	2,355	44	365	4
J	S	860	1,440	890	490	1,300	18	225	6
K	P	1,300	1,825	8,620	1,950	4,706	83	420	8
L	P	660	1,800	1,280	570	3,286	48	420	4
M	P	1,210	1,630	8,620	1,950	5,071	47	475	6
N	S	1,130	1,630	1,440	640	1,864	41	200	4
O	S	1,130	1,630	1,060	510	1,407	27	140	5
Q	P	1,445	1,630	870	410	1,999	46	140	7
R	P	861	1,700	1,440	640	3,231	44	340	4
S	P	914	1,700	750	370	1,595	35	230	5

RESULTS

Table 1 shows the dendrite lengths of thirty cells. In the second column, the letters P and S denote pyramidal and stellate cells respectively. The depths of the cells were measured from the pial surface to the lowest point of the perikaryon. The total cortical thickness is the distance from the pial surface to the grey-white boundary; this boundary is not sharp. The volumes and surfaces of the perikarya were estimated from measurements of heights and diameters by treating the perikarya as solids of revolution. The total lengths of the dendritic branches are the sums of the lengths of the individual branches; the figures have not been rounded off. The tangential extent of the dendrites of each neuron was measured under a low power and is the length of a straight line drawn between the extreme ramifications of the non-apical dendrites in a direction parallel to the pial surface.

An examination of the figures in column 4 shows a considerable variation in cortical thickness within any one area; this variation does not necessarily depend on any apparent change in curvature of the pial surface. It must be remembered also that any change in cortical thickness does not lead to a uniform displacement of cells at different depths (Bok, 1929); consequently, the recorded depths of the cells in column 3 must be regarded as a general guide to their positions.

The relationship between dendritic branches, depth of cell and volume of perikaryon

The correlation diagrams, Text-figs. 4–6, show that there is no simple relationship between the total number of branches or the total length of the dendrites and the depth or volume of the perikaryon. The most that can be said is that very deep and very large cells do not have few branches or very short dendrites. The only well correlated variables are total dendritic length and number of branches (Text-fig. 7).

Mean values

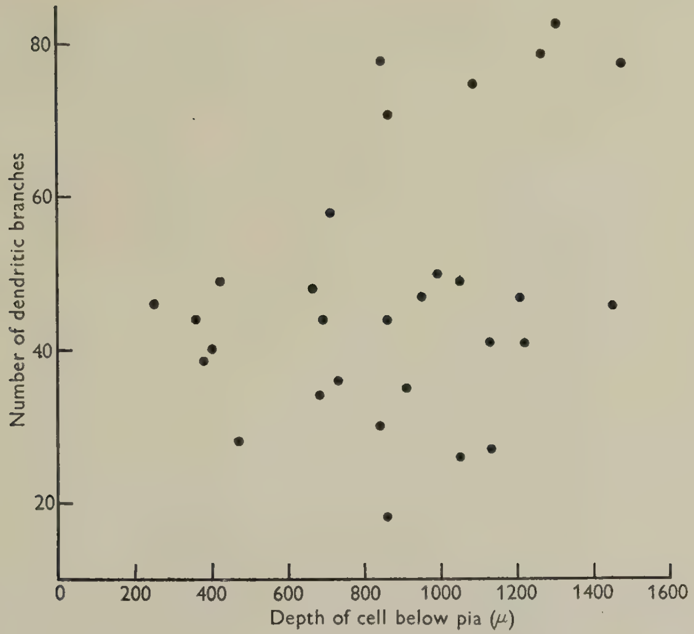
Mean values taken over such a heterogeneous set of data may be misleading and difficult to interpret, but it may be noted that the mean dendritic length in the visual area does not differ significantly from that found in the motor area (column 7), whereas the mean number of branches found in the visual area is somewhat higher than for the motor area.

Tangential extent of dendrites

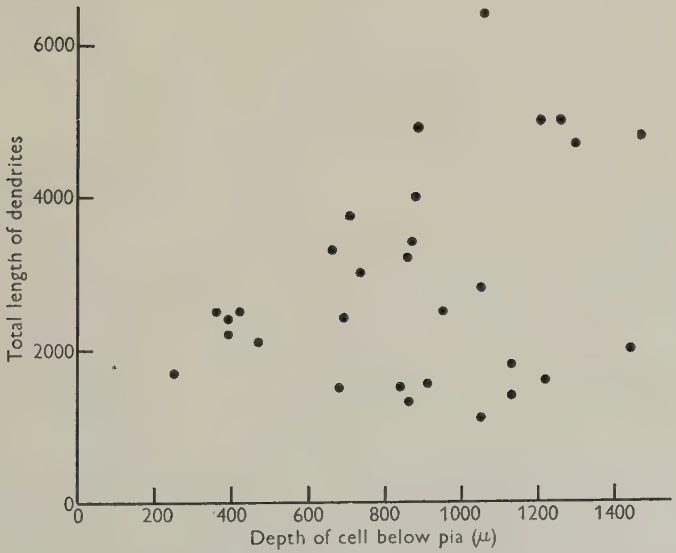
Column 9 shows that the extent of dendrites is large; usually more than 0·25 mm. and in several cases reaching 0·5 mm. The number of proximal branches is never less than four and seldom greater than seven, but there are examples both of stellate cells and pyramidal cells having more of these branches.

The manner of dendritic branching

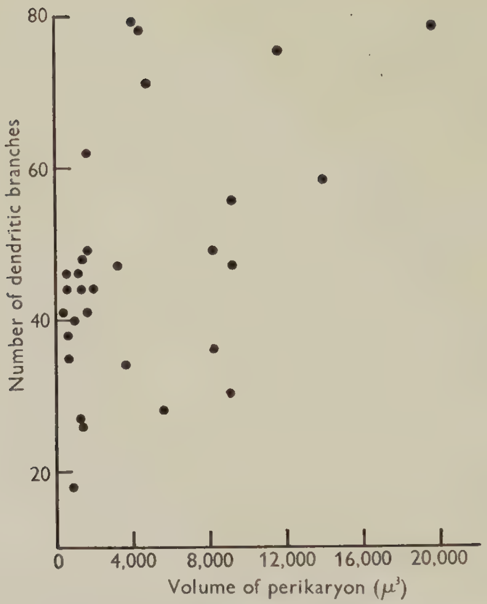
In order to study the way in which the number of branches varies with the distance from the perikaryon, it is convenient to use a series of concentric spherical shells as co-ordinates of reference. In the cases of the stellate cells and for the basal dendrites of pyramidal cells, these shells have their common centre in the perikaryon. Pyramidal cells have an apical ramification superimposed upon this basal distribu-



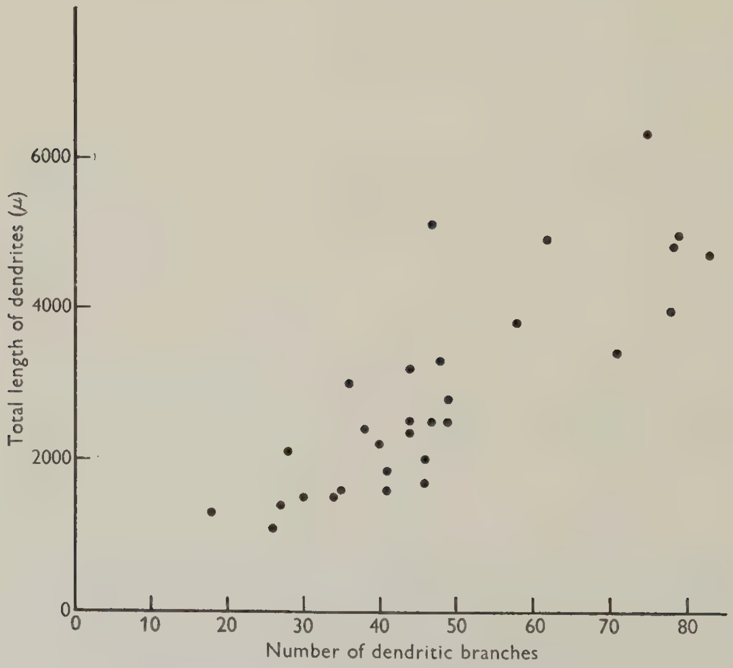
Text-fig. 4. Correlation diagram for the relationship between the number of dendritic branches and the depth of the cell below the pia.



Text-fig. 5. Correlation diagram for the relationship between the total length of dendrites and the depth of the cell below the pia.

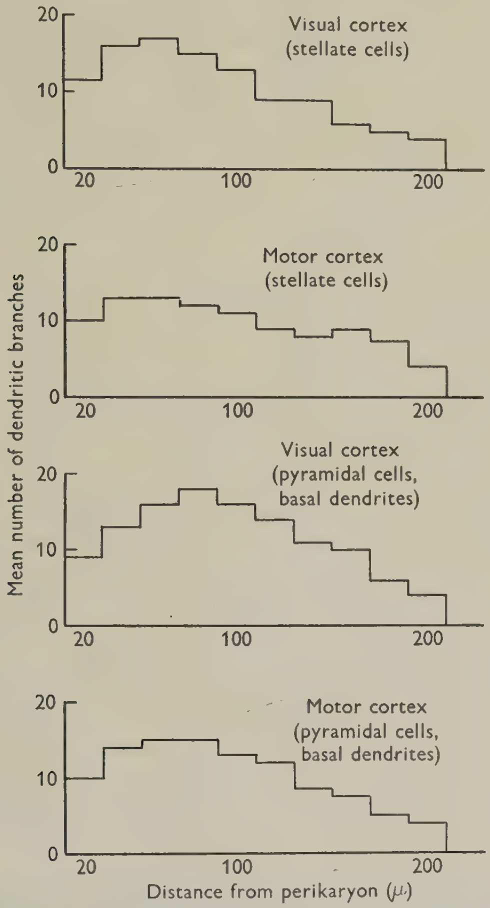


Text-fig. 6. Correlation diagram for the relationship between the number of dendritic branches and the volume of the perikaryon.



Text-fig. 7. Correlation diagram for the relationship between the total length of dendrites and the number of dendritic branches.

tion. The main pattern difference between the more superficial and the deeper lying pyramids lies in the length of the main, unbranched part of the apical dendrite; it is as if a terminal ramification were joined to the perikaryon by a 'stalk' of varying length. It is convenient, therefore, to refer the numbers of branches of this apical ramification to distances from the main bifurcation of the apical dendrite (Text-figs. 2 and 3).



Text-fig. 8. Histograms showing the distributions of numbers of dendritic branches with the distance from the perikaryon.

Tables 2 and 3 show the numbers of dendritic branches, together with their mean diameters, for the stellate cells and for the basal ramifications of the pyramids. These figures were compiled from graphs of the kind shown in Text-fig. 1. Four histograms have been drawn from the data of Tables 2 and 3, the stellate and pyramidal cells for the visual and motor areas being treated separately. The results are shown in Text-fig. 8; certain similarities and differences may be indicated.

It is clear that the distributions for both types of cells, whether stellate or pyramidal, from any one of the cortical regions are similar, while there is a difference between the distributions for the two different areas. The histograms for the visual

Table 2. *Number of branches (B) and mean diameters (D) of dendrites arising from stellate cells in the visual and motor cortices of the cat*

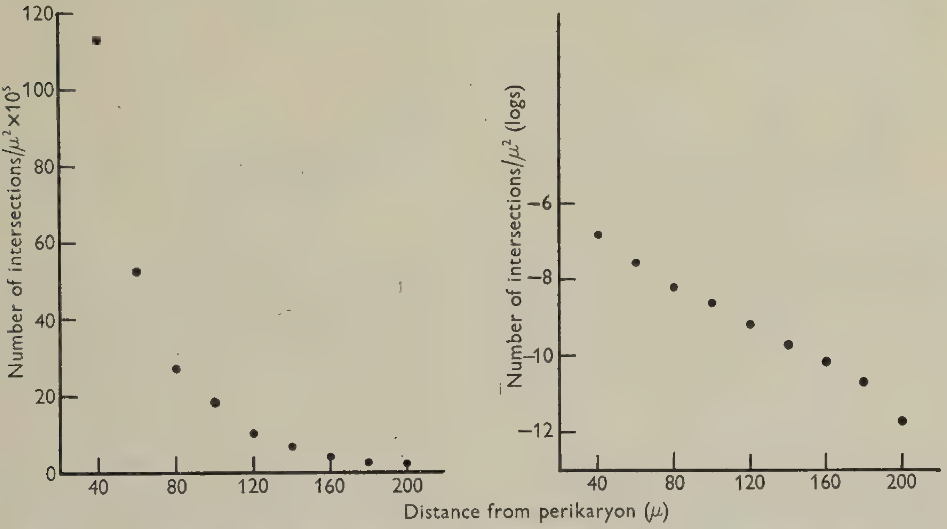
Cell	Distance from perikaryon (μ) 20		40		60		80		100		120		140		160		180		200		220		240	
	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D
											Visual													
8	18	2·0	22	1·5	23	1·5	21	1·4	22	1·3	18	1·3	15	1·3	12	1·2	9	1·1	4	1·0	2	1·0	1	1·0
15	6	1·4	8	1·0	10	0·8	10	0·6	7	0·6	7	0·6	7	0·6	1	0·5	—	—	—	—	—	—	—	—
20	10	2·2	15	2·3	14	1·4	12	1·4	10	1·4	6	1·4	5	1·4	2	1·8	2	1·8	2	1·8	1	1·0	—	—
22	16	1·2	13	1·0	14	1·0	10	1·1	6	1·0	5	0·8	4	1·0	2	1·0	—	—	—	—	—	—	—	—
23	11	2·6	21	1·5	22	2·0	22	1·3	21	1·3	15	1·0	12	0·9	12	0·5	10	0·8	9	0·8	6	0·8	4	0·8
											Motor													
E	12	1·9	18	1·4	20	1·3	23	1·1	22	1·1	17	1·0	15	1·0	10	1·0	9	1·0	8	1·0	6	0·9	3	0·8
F	11	2·2	13	1·8	13	1·7	10	1·7	9	1·9	8	1·9	9	1·6	8	1·7	5	2·0	5	2·0	4	2·1	3	1·7
H	14	2·6	15	1·7	12	1·7	10	1·5	10	1·3	10	1·3	11	1·2	11	1·2	10	1·3	9	1·3	12	1·0	4	1·0
J	8	1·5	10	1·3	9	1·1	7	1·4	8	1·0	7	1·1	3	1·5	2	1·8	2	1·8	—	—	—	—	—	—
N	7	2·1	11	1·5	12	1·4	13	1·3	9	1·2	7	1·2	7	1·3	10	1·2	7	1·3	—	—	—	—	—	—
O	8	1·8	11	1·4	13	1·2	11	1·2	9	1·2	6	1·3	5	1·4	3	1·8	2	2·0	—	—	—	—	—	—

Table 3. *Numbers of branches (B) and mean diameters (D) of basal dendrites arising from pyramidal cells in the visual and motor cortices of the cat*

	Distance from perikaryon (μ) 20		40		60		80		100		120		140		160		180		200		220		240	
	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D
											Visual													
6	7	2·9	10	2·7	17	2·1	17	1·8	14	1·9	12	1·9	11	1·9	7	2·1	4	2·3	—	—	—	—	—	—
9	15	3·0	22	2·6	23	2·2	26	2·0	25	1·9	19	1·8	15	2·1	10	2·1	8	1·8	7	1·9	7	1·9	4	2·3
10	8	3·7	12	2·7	15	2·1	22	1·8	22	1·5	20	1·4	16	1·4	13	1·3	10	1·3	6	1·4	4	1·4	4	1·4
11	14	3·4	21	2·6	26	2·1	25	2·1	21	2·0	16	2·0	14	2·0	11	1·9	9	1·7	6	1·8	4	1·8	3	2
12	7	2·6	13	2·5	15	2·3	16	2·2	18	2·1	14	1·8	11	2·0	11	1·5	8	1·6	7	1·6	3	2·3	—	—
13	7	1·6	8	1·1	7	1·1	5	1·4	4	1·3	3	1·2	4	0·9	—	—	—	—	—	—	—	—	—	—
21	5	3·7	8	2·7	12	2·1	12	1·9	16	1·8	13	1·6	8	1·3	7	1·3	4	1·2	—	—	—	—	—	—
											Motor													
A	9	3·8	15	2·6	15	2·2	17	2·1	20	1·8	19	1·6	18	1·6	18	1·5	16	1·5	13	1·6	11	1·8	8	1·9
B	7	1·9	10	1·7	11	1·7	13	1·5	13	1·3	14	1·4	10	1·4	10	1·3	6	1·2	—	—	—	—	—	—
C	11	1·7	14	1·4	16	1·3	16	1·3	13	1·3	8	1·6	5	1·7	5	1·7	4	1·9	3	2	3	2	—	—
D	8	2·3	14	1·4	13	1·2	10	1·1	9	1·1	8	1·0	4	1·1	4	1·1	—	—	—	—	—	—	—	—
G	11	1·7	12	1·5	13	1·3	13	0·9	13	0·9	11	0·9	10	0·9	8	1·0	6	0·9	4	0·8	4	0·8	3	0·7
I	7	3·1	15	1·8	18	1·5	11	1·4	9	1·4	8	1·5	7	1·6	6	1·7	4	1·9	3	2·2	—	—	—	—
K	11	3·9	20	2·4	24	2·1	23	2·2	19	2·3	22	2·1	13	1·7	8	1·8	4	1·5	5	1·4	3	1·3	—	—
L	7	2·9	14	1·9	14	1·8	15	1·7	15	1·4	15	1·4	11	1·4	9	1·3	6	1·3	6	1·3	3	1·5	4	1·5
M	11	3·0	10	3·0	12	2·2	12	2·2	12	2·1	11	2·0	9	1·9	10	1·7	9	1·5	7	1·5	7	1·5	4	1·6
Q	13	1·9	18	1·2	15	1·0	14	0·9	9	0·9	5	0·8	2	1·3	2	1·3	2	1·3	1	2·0	8	1·3	2	1·3

cortex show fairly sharp modal values, while those for the motor region tend to be more flattened with no clearly defined mode. Secondly, the mean numbers of branches are almost always uniformly less in the motor cortex than in the visual.

This branching problem can be approached in another way. Examination of a number of cells shows an apparently uniform distribution of dendrites around the perikaryon, the apical ramification being excluded for the present. There is no obvious asymmetry of dendrites, and although the precise manner of the distribution may vary, a general uniformity is maintained. If we assume this general uniformity of distribution, then the number of intersectons per unit area can be found from the known number of branches at any distance from the perikaryon by dividing this branch number by the area of the appropriate spherical shell (Text-figs. 2 and 3).

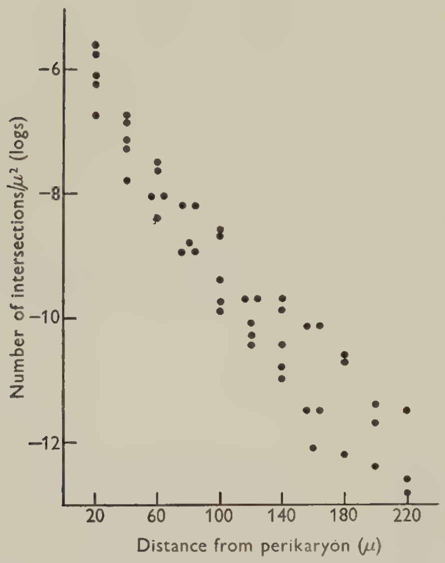


Text-fig. 9. Graphs showing the relationship between the number of intersections/unit area and the distance from the perikaryon for a single stellate cell.

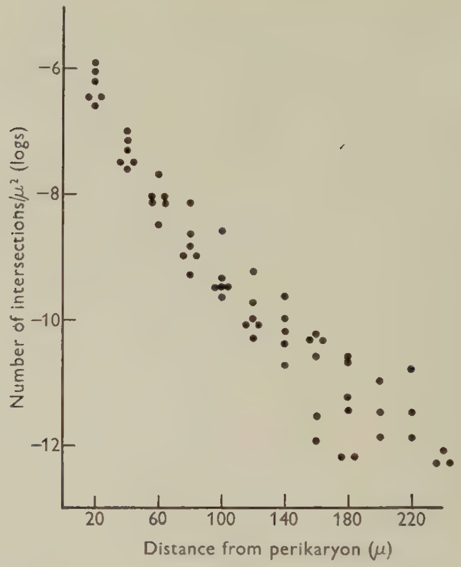
The results of this method applied to a single cell for increasing distances from the perikaryon are shown on the left-hand side of Text-fig. 9. This graph suggests that the mean number of intersections/unit area falls off exponentially with the distance. This is easily tested by plotting the logarithms of these numbers against the distance (Text-fig. 9, right-hand side) and the result is clearly linear. Complete linearity is evidently impossible since the number of intersections becomes zero, whereas the negative exponential function only approaches this value asymptotically.

Text-fig. 10 shows the results of plotting the logarithms of the numbers of intersections/unit area for all the stellate cells of the visual region. The general linearity is maintained, with the scatter increasing as more and more branches terminate. Similar results for the stellate cells of the motor area are shown in Text-fig. 11.

The graphs for the basal dendrites of pyramidal cells are shown on the left-hand sides of Text-figs. 12 and 13. In the latter figure, only the mean values are plotted in order to show the general trend; the line fitted to the points has, of course, been computed from all the observations and not from the mean values.



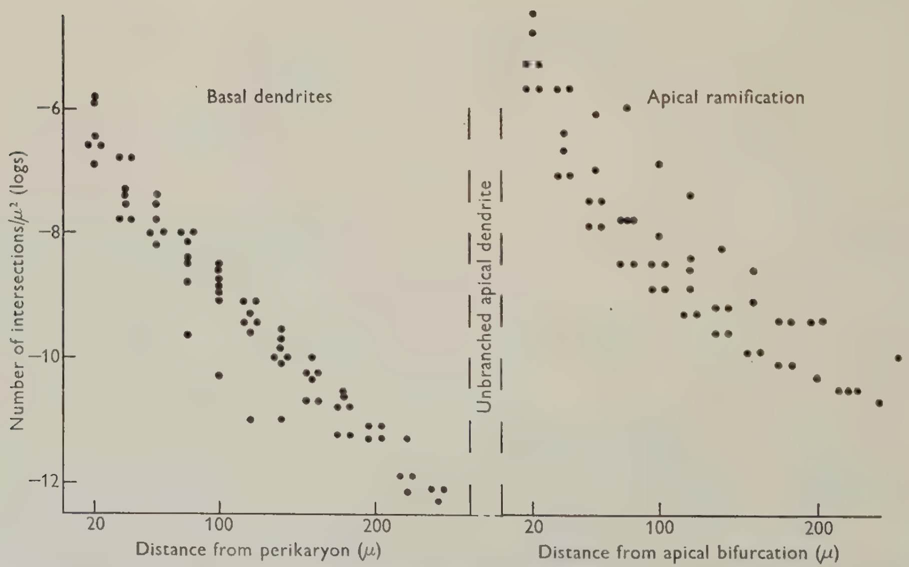
Text-fig. 10.



Text-fig. 11.

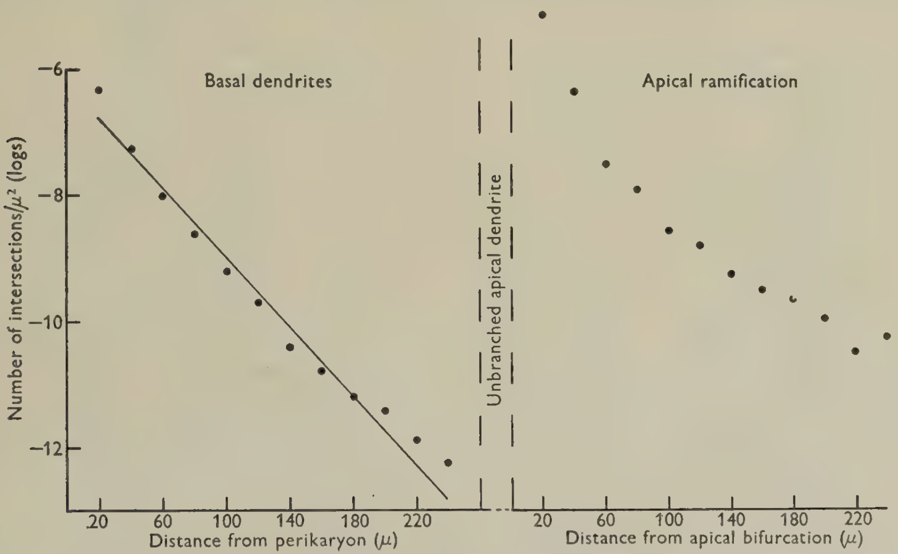
Text-fig. 10. Graph showing the relationship between the number of intersections/unit area and the distance from the perikaryon for stellate cells of the visual cortex.

Text-fig. 11. Graph showing the relationship between the number of intersections/unit area and the distance from the perikaryon for stellate cells of the motor cortex.



Text-fig. 12. Graphs showing the relationship between the number of intersections/unit area and the distance from the perikaryon for the basal dendrites and from the main apical bifurcation for the apical dendrites of pyramidal cells from the visual area.

In all these cases, lines have been fitted to the logarithmic form of $y = ae^{-kx}$, where a is a parameter of position and k of slope. The values for the parameter estimates are shown in Table 4. These results show significant differences between the slope of the line for the visual stellates and the slopes for the lines for the other cells. The numbers of dendritic branches of stellate cells from the visual cortex fall off more rapidly than the branches from other cells or, in other words, the numbers of dendritic intersections/unit area from pyramidal and stellate cells of the motor area and of pyramidal cells from the visual area fall off exponentially with distance from the perikaryon at the same rate, and this is lower than the rate for stellate cells of the visual cortex.



Text-fig. 13. Graphs showing the relationships between the mean number of intersections/unit area and the distance from the perikaryon for the basal dendrites and from the main apical bifurcation for the apical dendrites of pyramidal cells from the motor area.

Table 4. *Values of parameters for regression lines relating number of dendrites intersecting a unit area with distance from perikaryon*

(Natural logarithms throughout.)

Type of dendrite	Cortical area	Type of cell	Estimate of log a (estimated S.D.)	Estimate of k (estimated S.D.)
Non-apical	Visual	Stellate	−5·89 (0·82)	0·032 (0·002)
Non-apical	Motor	Stellate	−6·46 (0·58)	0·026 (0·004)
Non-apical	Visual	Pyramid	−6·30 (0·47)	0·026 (0·001)
Non-apical	Motor	Pyramid	−6·27 (0·52)	0·027 (0·001)
Apical	Visual	Pyramid	+1·22 (0·61)	2·09 (0·11)
Apical	Motor	Pyramid	+1·33 (0·51)	2·13 (0·09)

In the case of the non-apical dendrites the lines have been fitted to the logarithmic form of $y = ae^{-kx}$ ($\log y = \log a - kx$), while the logarithmic form of $y = ax^{-k} = ae^{-k \log x}$ ($\log y = \log a - k \log x$) has been used for the apical dendrites.

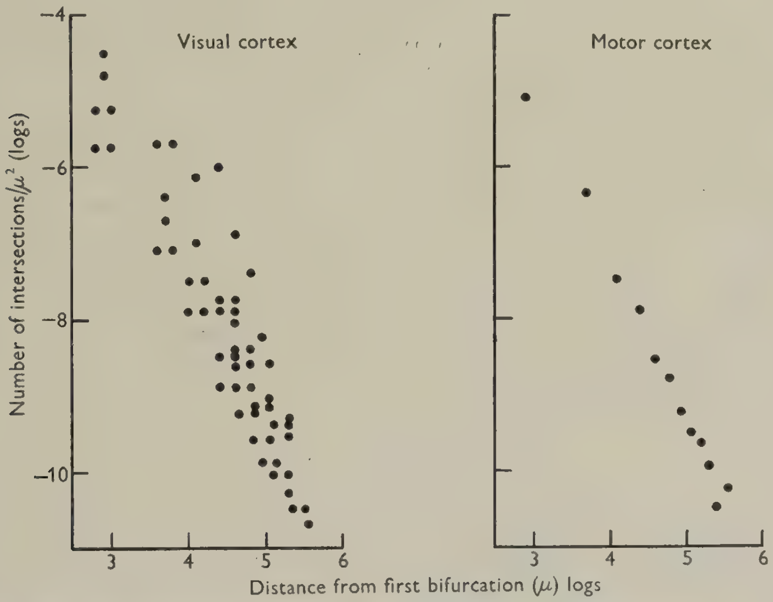
Table 5. *Numbers of branches (B) and mean diameters (D) of dendrites of the apical ramification of pyramidal cells from the visual and motor cortices of the cat*

	Distance from 1st apical bifurcation (μ)																							
	20		40		60		80		100		120		140		160		180		200		220		240	
	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D
	Visual																							
6	3	3·0	3	3·0	3	3·0	4	2·5	5	2·3	5	2·3	2	1·0	2	1·8	2	1·8	2	1·8	2	1·8	—	—
9	2	1·5	2	1·5	2	1·5	2	1·5	2	1·5	2	1·5	3	1·5	—	—	—	—	—	—	—	—	—	—
10	2	0·5	2	0·5	2	0·5	2	0·5	2	0·5	2	0·5	2	0·5	—	—	—	—	—	—	—	—	—	—
11	3	2·3	4	1·8	3	1·7	4	2·3	3	2·3	3	2·3	3	2·3	4	1·8	4	1·8	5	1·4	2	2·5	—	—
13	7	1·6	8	1·1	5	1·4	4	1·3	3	1·2	4	0·9	—	—	—	—	—	—	—	—	—	—	—	—
21	5	3·7	8	2·7	12	2·1	12	1·9	16	1·8	13	1·6	8	1·3	7	1·3	4	1·2	—	—	—	—	—	—
	Motor																							
A	4	1·6	3	1·4	3	1·4	3	1·2	1	2·0	1	2·0	1	2·0	2	1·3	2	1·3	2	1·3	2	1·3	2	1·3
B	6	1·1	6	0·9	4	1·0	3	1·0	3	0·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C	3	2·3	3	2·3	1	0·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
D	2	1·0	3	0·8	3	0·8	3	0·8	2	0·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K	5	2·7	5	2·7	4	2·6	5	2·2	6	2·0	5	2·1	3	2·5	4	1·8	4	1·8	5	1·4	4	1·5	4	1·3
L	3	1·0	3	1·0	4	0·8	3	0·7	4	0·6	5	0·6	5	0·6	2	0·5	—	—	—	—	—	—	—	—
M	5	0·8	5	0·8	5	0·8	5	0·8	5	0·8	5	0·8	4	1·0	4	1·0	4	1·0	4	1·0	4	1·0	4	1·0

Table 6. *Cell densities at varying depths in the visual cortex of the cat*

	Depth below pial surface (μ)															
	0–150	151–250	251–350	351–450	451–550	551–650	651–750	751–850	851–950	951–1050	1051–1150	1151–1250	1251–1350	1351–1450	1451–1550	1551–1650
No. of cells/$10^6\mu^3$	Very few	104	84	76	64	57	60	69	67	56	66	76	82	79	70	60

If we now turn to the apical ramification (Table 5), where the distances have been measured from the main bifurcation, the state of affairs is quite different and is shown by the graphs on the right-hand sides of Text-figs. 12 and 13. These graphs confirm the impression formed from qualitative observation, that the apical dendrites do not terminate as fast as other dendrite branches. Logarithmic plotting (Text-fig. 14) shows that one may consider the relationship to be of the form $y=ax^{-k}$ (i.e. $y=ae^{-k\log x}$), and in this case the exponential decay falls off with $\log x$ instead of with x. This slower rate of decay is found in both the motor and the visual areas. It may be said that there is no difference between the branching patterns of the apical dendrites of cells from these two areas.



Text-fig. 14. Graphs showing the double logarithmic plotting of data shown on the right of Text-figs. 12 and 13.

The packing density of perikarya

The packing density or number of perikarya contained in a unit volume of cortex can be found from Nissl preparations by counting the number of cells at different cortical depths in known volumes of tissue; from these numbers the mean distances between cells at different depths can be estimated. This work has been carried out extensively for the human brain (van Alphen, 1945). Complete figures for the cat are not yet available, but Table 6 gives the mean values for the number of cells contained in $10^6\mu^3$ (10^{-3} mm.^{3}) found in the cat visual cortex at different depths. These figures are based on counts with seven replications made by Miss C. Blockley. For the region in the neighbourhood of Gennari's line this mean number is 60.

Since the average volume of such perikarya is $4000\mu^3$, it is clear that the perikarya occupy about 25% of the cortex by volume. Again, since sixty perikarya are contained in $10^6\mu^3$ of cortex, the volume of cortex available to each perikaryon is

$10^6/60\,\mu^3 = 16 \times 10^3\,\mu^3$. The cube root of this number is approximately equal to 25 and we may imagine each perikaryon to be placed at the centre of a cube of side $25\,\mu$ with a distance of about $25\,\mu$ between the centres of neighbouring perikarya.

Consider now a stellate cell with its dendrites extending throughout a cortical sphere of radius $200\,\mu$. The volume of this sphere will be $\frac{4}{3}\pi \times 200^3\,\mu^3$, i.e. about $32 \times 10^6\,\mu^3$, and hence it will contain about $60 \times 32 = 2000$ cells; a cell with a dendritic field of $250\,\mu$ will have about 4000 cells within its dendritic field.

The packing density of the dendrites

The dendritic population of any region of the cortex will be formed from the contributions of a number of cells, the nearby cells making up the major part of the total. If the concentric shell method is used again, the number of intersections made by the dendrites at different distances is known and, with a knowledge of the numbers of cells between the pairs of consecutive shells, the number of dendrites cutting a small area, say $100\,\mu^2$, of cortex may be estimated. For example, in the Gennari region, and fixing a point, it is found that, within a radius of $20\,\mu$ there are an average of two perikarya, each of which will contribute 0·15 intersections per $100\,\mu^2$; between the $20\,\mu$ shell and the $40\,\mu$ shell there are 13 perikarya contributing 0·08 intersections per $100\,\mu^2$ and so on. As a result we find that such a thin sheet of cortex will have a mean number of nine intersections. The mean dendritic diameter is $1{\cdot}5\,\mu$; and, consequently, the total area of dendritic sections will be $9\pi \times 0{\cdot}56\,\mu^2$, i.e. about 18 % of the cortex. If we again consider a square sheet of cortex of side $10\,\mu$ with the dendrites uniformly spaced, it is clear that there will be a distance of the order 2–3 μ between their surfaces.

The connective zone of a neuron

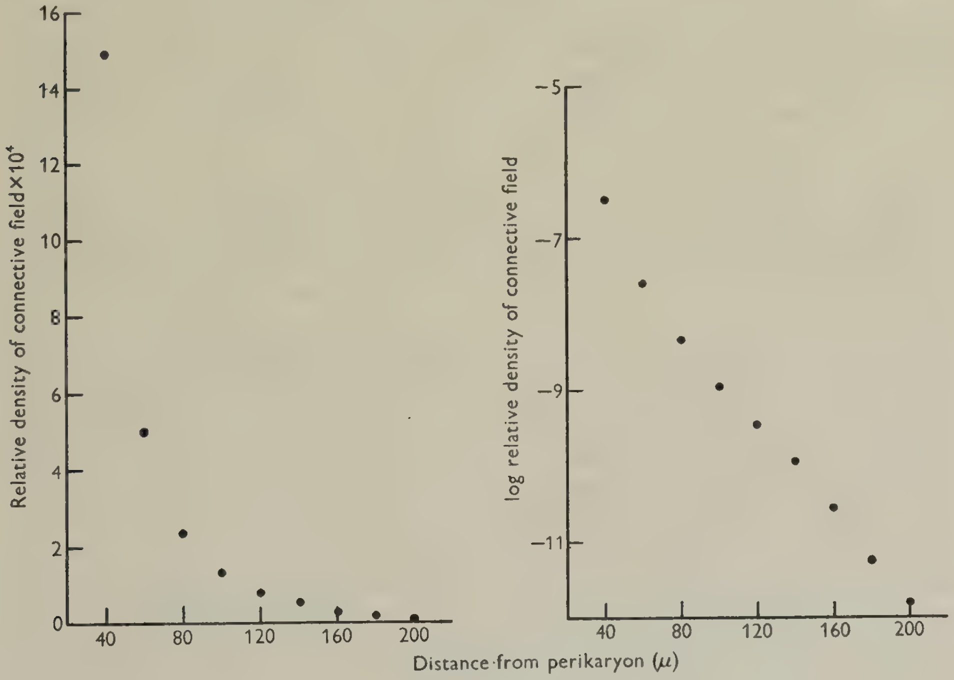
Knowledge of the organization of cortical synapses is still fragmentary, and the conditions under which effective connexions are made unknown. It is, however, possible to arrive at some rather general conclusions about the region that will be called the connective zone of the neurone.

Table 7. *Density of connective field of a neuron at increasing distances from the centre of its perikaryon*

Distance (μ)	Target volume (μ^3) (T)	Total volume (μ^3) (V)	Density of connective field (T/V)	Natural logarithm of density
20	1008	33,510	0·030080	−3·50
	(282)*	33,510	(0·008420)	(−4·78)
40	350	234,570	0·001490	−6·51
60	315	636,680	0·000495	−7·61
80	292	1,239,850	0·000236	−8·35
100	263	2,044,090	0·000129	−8·96
120	236	3,049,370	0·000077	−9·47
140	205	4,255,720	0·000048	−9·94
160	145	5,663,120	0·000026	−10·57
180	113	8,830,300	0·000013	−11.27
200	69	9,081,100	0·000008	−11.79

* Target due to perikaryon excluded.

The perikaryon and dendrites of a cell may be imagined to be surrounded by a zone with a thickness equal to the radius of a fine axonal branch, say $0{\cdot}5\,\mu$. Any branch lying within this connective zone may be said to 'make a contact' with the neuron. The region between any consecutive pair of the spherical shells that have been previously considered will have a total volume V and contain a connective zone of volume T; these quantities may be computed from the data, and the value of the ratio T/V will be called the density of the connective field for this region. A set of values for this ratio T/V is given in Table 7 for a stellate cell from the Gennari region and corresponding graphs in Text-fig. 15. Such graphs show that the value of the density of the connective field falls off exponentially with the distance from the perikaryon; in the case illustrated, the slope of the fitted line is −0·035. This exponential relationship appears to hold well except for the region adjacent to the perikaryon, where the connective zone is enormously increased as a result of the presence of the perikaryon itself.



Text-fig. 15.

DISCUSSION

In the early stages of a study of dendrites in the cerebral cortex the observer is at first bewildered by the extent and complexity of the ramifications of these dendrites but, later, he becomes aware that there are certain common features among the dendritic patterns of the various neurons and certain differences as well. These similarities and diversities are difficult to specify qualitatively, and only quantitative methods serve to make them clear. Bok (1936) appreciated this situation, and made the only available previous quantitative study of dendritic branching. However, he measured only eight cells from the outer layers of an unspecified part of the cortex and was mainly concerned with a possible indirect relationship between the number of dendritic branches and the nuclear volume of the cell.

At first sight it would appear reasonable to assume that there would be simple relationships between the numbers of branches and the size of the perikaryon or the depth of the cell within the cortex. The correlation diagrams show that this is not the case; in general, the correlations are low and no simple relationships of these kinds appear. The only simple relationship is that which shows that the number of dendritic branches is proportional to the total dendritic length (Text-fig. 9), a fact which suggests that each branch, as it grows longer, has an increasing tendency either to form a new pair of branches or to terminate.

These facts become a little clearer when the histograms of Text-fig. 8 are examined. It has been noted earlier that the numbers of branches in the cells of the visual cortex appear to show sharper modes in the visual cortex than those of the motor area and, in addition, the numbers of branches in the neighbourhood of the perikaryon are also greater in the visual area. These histograms must also be considered together with another aspect of the branching illustrated in the graphs showing the intersections per unit area. The slopes of the fitted lines are a measure of the rate of decay of the numbers of branches with distance from the perikaryon; this rate is the same for all the cells considered, apart from the stellate cells of the visual area and in that case the rate is significantly higher. Altogether these results suggest that these latter cells have a relatively small field of interaction, and it must be remembered that they are associated with the terminations of the axons arriving from the lateral geniculate body and that many of them have short many-branched axons.

The contrast between the basal and the apical branching patterns shows that, in the apical ramification, the tendency for a branch to bifurcate rather than to end is greater than amongst the basal dendrites; this is combined with a tendency for all branches to terminate within a comparatively small volume near the pial surface, whereas the basal dendrites branch within a more diffuse local field of axons, infiltrating a more extended spherical volume.

A definition has been suggested for the connective zone of a neuron as a thin sleeve surrounding the perikaryon and dendrites. Furthermore, it has been shown that each neuron has a connective field associated with it in such a way that the density of this field falls off exponentially with the distance from its centre.

Various analogies with known computing mechanisms and switching devices have been suggested in order to account for that pattern discrimination which is believed to be one of the activities of the cortex. None of these models has shown any very striking agreement with our limited knowledge of the organization of the cortex and no guidance for future research has resulted from their description. In collaboration with Dr A. M. Uttley, an attempt has been made to consider the possibilities of a model that should have at least some features in common with this part of the cortex; the basic principles of such a machine have been discussed elsewhere, and it has been shown that it is possible to design and construct a model in accordance with these principles (Sholl & Uttley, 1953). A machine designed to such a specification has certain features: (1) the connexions between the inputs and the units can be random, i.e. only describable in terms of probabilities; (2) the machine can have limited storage; (3) all the units are identical. This machine is able to measure pattern differences by comparing a new presentation with a stored statistical ensemble of past presentations and operates entirely on a probability basis.

A study of the theoretical principles of the machine leads to the conclusion that, for a machine of this type, the probability of connexion between an input at a given position relative to a unit is a function of that position; this function defines a probability connective field whose density falls off exponentially with the distance from the unit. The manner in which the density of the connective field of the neuron decays with distance suggests that it may be interpreted as a probability connective field of this kind. Moreover, although further knowledge will lead to a modification in the values of the parameters found for the connective probability field in cortical neurons, it appears that the form of the law has been established.

It would be hazardous to press this analogy too far at this stage, but it is of interest to note that if the stellate cells of the Gennari region are compared with the units of this machine, then the afferent endings may well ramify among these cells in a random way, in the sense that two axons entering the visual area side by side are as likely to terminate on rather widely separated cells as on neighbouring cells.

SUMMARY

1. The sizes and arrangement of the dendrites of neurons in the visual and motor areas of the cerebral cortex of the adult cat have been studied quantitatively in Golgi-Cox preparations.

2. There is no simple relationship between the number of dendritic branches and the depth of the cell below the pial surface or with the volume of the perikaryon of the cell of origin.

3. The dendrites of stellate cells and the basal dendrites of pyramidal cells may extend to a distance of 0·25–0·5 mm. around the perikaryon. The region infiltrated by the dendrites of one cell contains between 2000 and 4000 perikarya.

4. The distributions of the numbers of dendritic branches of neurons at increasing distances from the perikarya in the visual cortex show a sharp modal value about 60–80μ from the perikarya. Comparative histograms for the motor cortex are generally flatter, with no sharp mode.

5. At corresponding distances from the perikarya, the numbers of dendritic branches in neurons from the visual cortex are generally greater than in the motor cortex.

6. The number of branches of dendrites of stellate cells and of the basal dendrites of pyramidal cells crossing a unit area falls off exponentially with the distance from the perikaryon. This rate of decay is higher in the stellate cells of the visual cortex than in any other type of cell studied.

7. The number of branches in the apical ramification of pyramidal cells falls off more slowly, the logarithms of the branch numbers falling off with the logarithm of the distance.

8. Studies of Nissl preparations show that, in the visual area, 25–35% of the cortex is occupied by perikarya while, from the complementary explanation of both Nissl and Golgi-Cox preparations, it was found that the dendrites in this region occupied 20–30% of the cortical volume.

9. In the region of Gennari's line, the mean spacing between the centres of perikarya is 25μ, while the mean distance between the surfaces of neighbouring dendrites is 2–3μ.

10. A connective zone and a connective field are defined for a neuron. It is shown that the density of the connective field falls off exponentially with distance from its centre.

11. An analogy between the visual cortex and a statistical machine with a random input is considered.

I should like to thank Prof. J. Z. Young, not only for his continued encouragement, but for the constructive criticism that has enabled work which tended to be diffuse to be brought to a focus. Dr R. Lorente de Nó has given me the benefit of his unrivalled knowledge of cortical histology while many conversations with Mr B. G. Cragg and Dr A. M. Uttley have been most helpful. I am grateful to Mr G. Hyde for his valuable assistance on the technical side. The plates are from some of the last photographs made by the late Mr F. J. Pittock, F.R.P.S.

REFERENCES

Alphen, G. W. H. M. van (1945). *Kernmetingen in de Neocortex van de Mens.* Leiden: Eduard Ijdo.

Bok, S. T. (1929). Der Einfluss der in den Furchen und Windungen auftretenden Krümmungen der Grosshirnrinde auf die Rindenarchitektur. *Z. ges. Neurol. Psychiat.* **121**, 682–750.

Bok, S. T. (1936). The branching of the dendrites in the cerebral cortex. *Proc. Acad. Sci. Amst.* **39**, 1209–1218.

Lorente de Nó, R. (1922). La corteza cerebral del ratón. *Trab. Lab. Invest. biol. Univ. Madr.* **20**, 41–78.

Lorente de Nó, R. (1933). Studies on the structure of the cerebral cortex. I. The area Entorhinalis. *J. Psychol. Neurol., Lpz.*, **45**, 381–438.

Lorente de Nó, R. (1934). Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *J. Psychol. Neurol., Lpz.*, **46**, 113–177.

O'Leary, J. L. (1941). Structure of the area striata of the cat. *J. comp. Neurol.* **75**, 131–164.

Ramón y Cajal, S. (1911). *Histologie du système nerveux de l'homme et des vertébrés*, vol. 2. Paris: A. Malome.

Sholl, D. A. & Uttley, A. M. (1953). Pattern discrimination and the visual cortex. *Nature, Lond.*, **171**, 387–388.

EXPLANATION OF PLATE

Plate 1

Fig. 1. Pyramidal cell from visual cortex of adult cat. Preparation as in Fig. 2. Unretouched photograph by double exposure.

Fig. 2. Stellate cell from visual cortex of adult cat. Golgi-Cox stain with sulphite-oxalic reduction and cut at 160μ. The axon runs towards the right-hand bottom corner of the photograph to the left of a large dendrite. Several axonal branches can be seen.

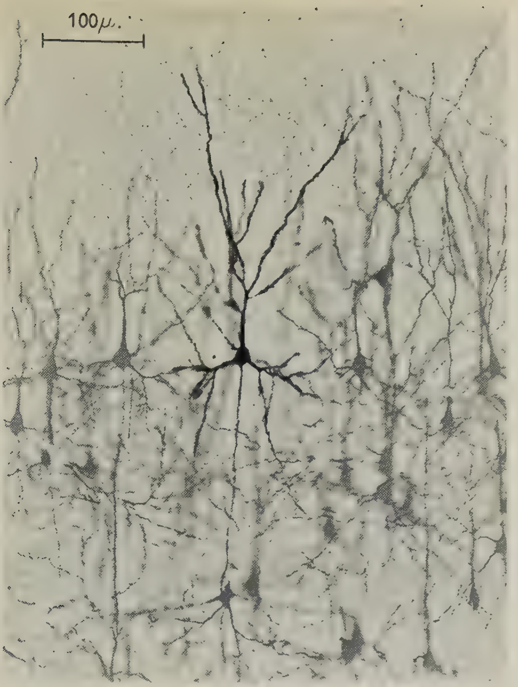


Fig. 1

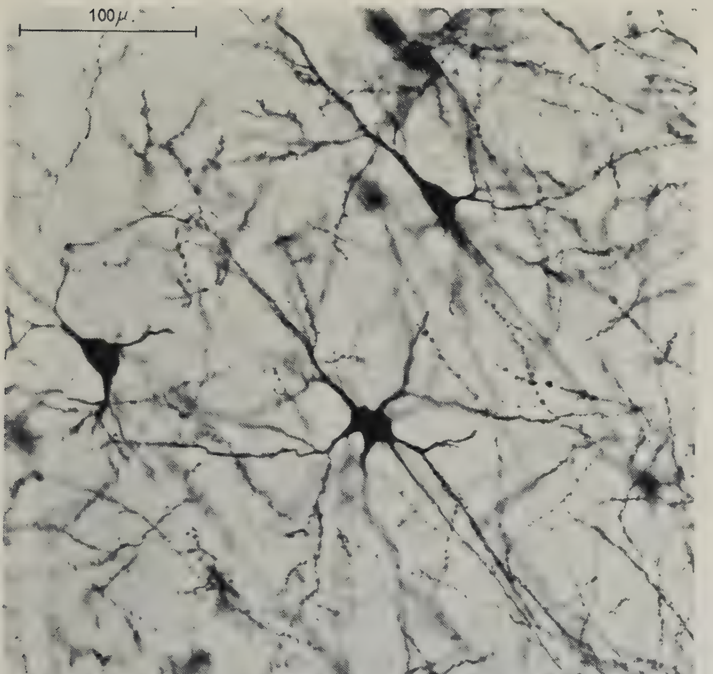


Fig. 2

SHOLL—Dendritic organization in cortical neurons

THE AMPLITUDE OF FOREARM AND OF HUMERAL ROTATION

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Because of the apparent lack of experimentally derived data on the amplitude of pronation and supination and of humeral rotation, it was decided to determine the amplitude of these movements with the elbow flexed and extended on a range of normal subjects. This is an extension of a previous study (Darcus & Salter, 1953).

A review of figures given in the literature for the amplitudes of total rotation of the upper limb (Table 1), humeral rotation (Table 2) and pronation and supination with the elbow flexed (Darcus & Salter, 1953) reveals the unsatisfactory state of the knowledge of this aspect of joint mechanics. The majority of the values given for

Table 1. *Average amplitude of total rotation of upper limb as reported by different authors*

Source	Amplitude (°)
Brash & Jamieson (1943)	360 (including scapular movement)
Johnston & Whillis (1946)	270
Mainland (1947)	<315 (including scapular movement)
Appleton *et al.* (1948)	*c.* 270
Darcus (1951) (mean of 10 readings on the right side of each of 3 male subjects)	255 (arm adducted) 253 (arm flexed 90°) 268 (arm abducted 90°)

Table 2. *Average amplitude of humeral rotation as reported by different authors*

(Lateral and medial rotation are given from a neutral position in which the forearm is at right angles to the longitudinal axis of the body)

	Joint position		Average amplitude (°)		
	Elbow	Shoulder	Lateral	Medial	Total
Macalister (1889)	—	—	—	—	97
Bryce (1915)	—	—	—	—	*c.* 90
Frescoln (1929)	Flexed 90°	—	40	50	90
Silver (1923)	Flexed 90°	Abducted 90°	*c.* 90	*c.* 90	*c.* 180
Dorinson & Wagner (1948)	Flexed 90°	Abducted 90°	90	90	180
Glanville & Kreezer (1937) (3 observations on 10 subjects)	Flexed 90°	Abducted 90°	R. 83 L. 83	94 100	177 183
Rosen (1922)	Flexed 90°	Adducted	45	—	—
Johnston & Whillis (1946)	Extended	—	—	—	100–110
Mainland (1947)	Extended	—	—	—	*c.* 180
Appleton *et al.* (1948)	Extended	—	—	—	100–120
Clark (1920)	Extended	—	—	—	130

the amplitude of humeral rotation with the elbow extended seem to have been obtained by deducting the amplitude of pronation and supination with the elbow flexed from that of total rotation with the elbow extended. Clark (1920) appears to be the only investigator who has attempted to measure this movement directly. No information has been found concerning the relative degrees of movement at the gleno-humeral joint, and at the radio-ulnar joints in total rotation of the upper limb, or the amplitude of radio-ulnar movement with the elbow extended.

To avoid any ambiguity arising through the use of the terms pronation and supination, the movements at the radio-ulnar joints alone are being described as forearm rotation.

METHODS

The wrist-cuff arthrometer (Darcus & Salter, 1953) was used to measure the amplitude of forearm rotation with the elbow flexed to 90° and the shoulder adducted and the amplitude of total rotation with the forearm extended and the shoulder flexed to 90° (Fig. 1).

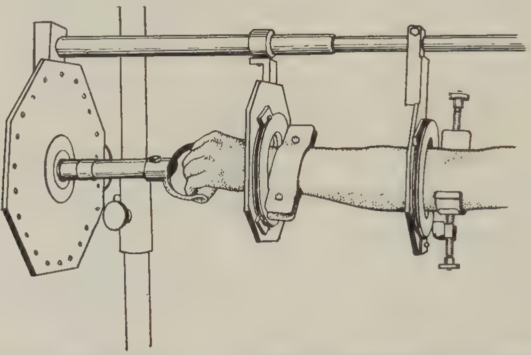

Fig. 1. The hand-grip, wrist-cuff and elbow-cuff arthrometers in position for simultaneous recording of amplitudes of rotation of the upper limb at different levels.

The main features of the apparatus designed to measure humeral rotation with the elbow extended are shown in Figs. 1 and 2. The attachment of the epicondyle clamps to the recording scale was allowed to slide to a limited degree in a plane parallel to the line between the medial and lateral epicondyles to compensate for the difference between the radius of the arc of movement of the two epicondyles when the limb is rotated. This difference is due to the axis of this movement passing closer to the lateral than to the medial epicondyle.

The apparatus for measuring humeral rotation with the elbow and the shoulder flexed to 90° is shown in Fig. 3.

For each of the movements, the total amplitude was measured in most cases on both right and left sides. Where possible, the results have been analysed into part movements from a neutral position. For the forearm rotation with the elbow flexed and for total rotation this position was with the distal ends of the radius and ulna in the same vertical plane. For humeral rotation with the elbow flexed the neutral position was with the forearm vertical.

The general experimental procedure was the same as described in the previous paper (Darcus & Salter, 1953).

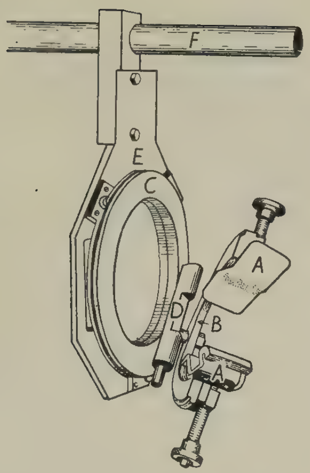

Fig. 2.

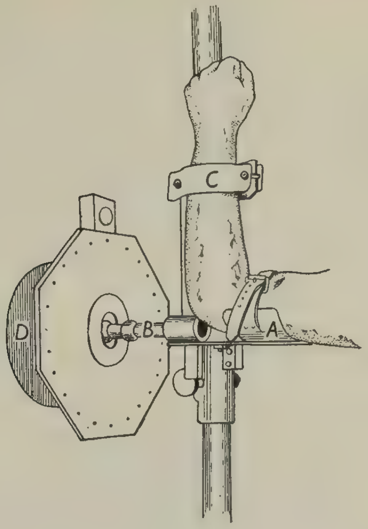

Fig. 3.

Fig. 2. The elbow-cuff arthrometer consists of two rubber-lined shaped metal plates (*A*) which can be clamped on the medial and lateral humeral epicondyles. These are joined by a U-shaped bar of metal (*B*) which is attached to a circular scale (*C*) through a joint (*D*) allowing movement on the same plane as the epicondyle clamps. The scale rotates in a metal frame (*E*) which is suspended from a tubular metal rod (*F*) along which it can be moved.

Fig. 3. The apparatus for measuring humeral rotation with the elbow flexed. A rubber-lined trough (*A*) fixed to the spindle of the apparatus (*B*) and in line with it, supports the distal end of the arm. The forearm is held in a rubber-lined metal clamp (*C*) proximal to the wrist. This clamp is attached by a metal bar to the spindle. The back of the measuring scale can be seen (*D*).

Table 3. *A comparison of the amplitude of total rotation of the upper limb and of humeral rotation recorded under different experimental conditions*

(Each figure is the average of fifty readings)

		Average amplitude (°)	
		Subject 1	Subject 2
(i) Total rotation using wrist-cuff arthrometer	(*a*) With elbow-cuff arthrometer in position	242·2	256·0
	(*b*) Without elbow-cuff arthrometer	250·4	261·5
(ii) Humeral rotation using elbow-cuff arthrometer	(*a*) With wrist-cuff arthrometer in position	110·7	125·8
	(*b*) Without wrist-cuff arthrometer	106·0	124·3
(iii) Total rotation using hand-grip arthrometer	(*a*) With wrist-cuff arthrometer in position	295·9	278·4
	(*b*) With elbow-cuff arthrometer	297·0	278·5
	(*c*) With both in position	297·5	277·3
	(*d*) With neither	289·6	278·7

THE EFFECT OF THE ARTHROMETERS ON THE AMPLITUDE OF MOVEMENT

To determine whether the recording devices interfered with the movements measured, a comparison was made of the amplitudes of total rotation of the upper limb and of humeral rotation with the elbow extended and the arm flexed to 90° with and without the wrist-cuff and elbow-cuff arthrometers in position. Ten consecutive measurements were made on the right side of two subjects on five consecutive days.

The results are summarized in Table 3. An analysis of the complete data indicates that no part of the apparatus had a significant effect on comparable groups of readings. In the previous study, it was determined that the wrist-cuff arthrometer did not restrict forearm rotation when the elbow is flexed (Darcus & Salter, 1953).

THE MEASUREMENT OF THE AMPLITUDE OF FOREARM AND HUMERAL ROTATION WITH THE ELBOW FLEXED AND OF HUMERAL ROTATION AND COMBINED FOREARM AND HUMERAL ROTATION WITH THE ELBOW EXTENDED

Twenty male and five female subjects were used, whose ages ranged from 16 to 38 years. All were right-handed. Ten readings were taken on both right and left sides of forearm rotation with the elbow flexed to 90° and the arm adducted, of humeral rotation with the elbow flexed and the arm flexed to 90° in the sagittal plane, and of humeral rotation and of combined forearm and humeral rotation with the elbow extended and the arm flexed to 90°. The amplitude of forearm rotation with the elbow extended was taken as the difference between the amplitude of the combined rotation and that of humeral rotation with the elbow extended.

As the amplitudes of forearm rotation with the elbow flexed and extended were not strictly comparable, owing to the different positions of the arm in which they were recorded, a subsidiary experiment was made in which measurements were taken on four subjects of forearm rotation with the elbow flexed and the arm adducted, and with the elbow flexed and the arm flexed to 90°.

RESULTS (Table 4)

Forearm rotation (Fig. 4). The analysis of the results of the subsidiary experiment indicates that there is no significant difference between the amplitude of forearm rotation with the elbow flexed in the two positions of the arm (Table 5). It was therefore decided that the amplitude of forearm rotation with the elbow flexed and extended could be directly compared.

The average amplitude of forearm rotation with the elbow flexed was 151° and with it extended 113°. The reduction was found on both sides in all subjects, and in forty-five of the fifty values it exceeded 15 % of the amplitude with the elbow flexed.

Humeral rotation (Fig. 5). In this case, no consistent effect of elbow position on amplitude was evident. On the average, the amplitude was greater with the elbow flexed (142°) than extended (135°), and this was also true of the majority (31/50) of the individual groups of readings. The degree of the differences was also variable, but in thirty-two of the fifty readings they were less than 10 % of the amplitude with the elbow flexed.

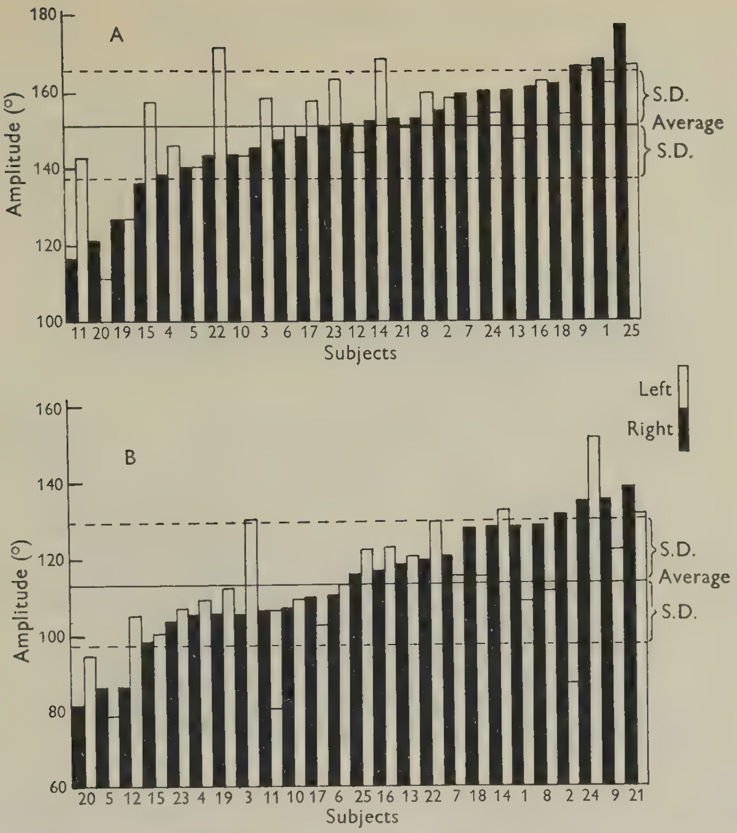

Fig. 4. Histograms of the average amplitude of forearm rotation for each subject on the right and left sides (A) with the elbow flexed and (B) with it extended. The average and standard deviation for all subjects is indicated. The order is determined by the amplitude on the right side in each subject.

Table 4. *The summarized results of the experiments on the amplitude of forearm and humeral rotation*

	Side						Difference between right and left as percentage of right	
	Right		Left		Both			
	Av.	S.D.	Av.	S.D.	Av.	S.D.	Av.	S.D.
Forearm rotation								
Elbow flexed	150	14·5	153	13·5	151	13·9	−2	8·2
Elbow extended	114	15·9	113	16·9	113	16·2	—	—
Percentage difference*	—	—	—	—	25	9·5	—	—
Humeral rotation								
Elbow flexed	139	11·2	144	13·1	141	12·3	−4	7·8
Elbow extended	134	16·0	135	15·1	135	15·4	−1	11·5
Percentage difference*	—	—	—	—	4	13·5	—	—
Total rotation	248	22·9	248	24·8	248	23·6	0	7·3
Percentage humeral rotation of total rotation	54	4·5	55	4·2	54	4·3	—	—
Total rotation − forearm rotation with elbow flexed	98	18·5	95	19·1	97	18·6	—	—
Percentage difference*	—	—	—	—	62	13·1	—	—

* The amplitude with the elbow flexed ≡ 100.

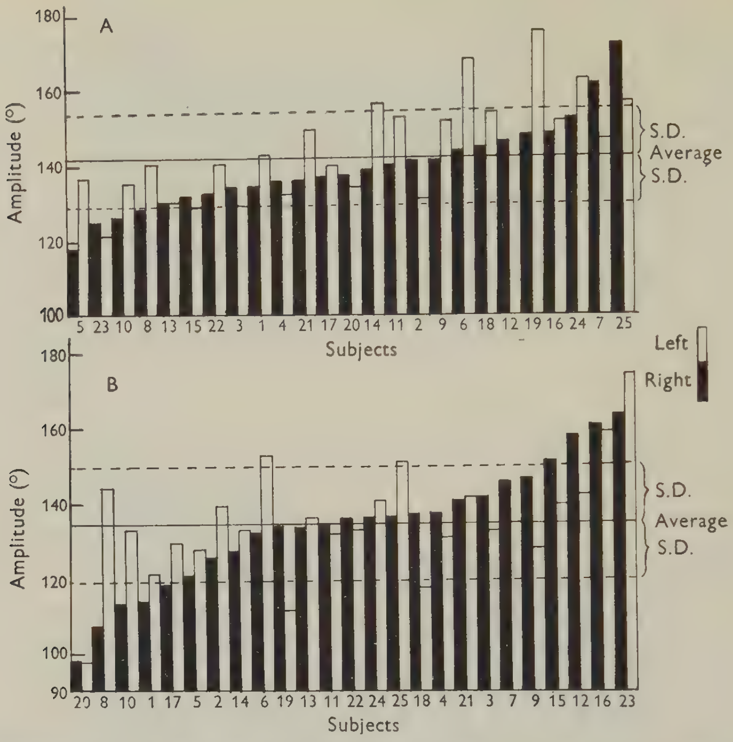

Fig. 5. Histograms of the average amplitude of humeral rotation for each subject on the right and left sides (A) with the elbow flexed, and (B) with it extended. The average and standard deviation for all subjects is indicated. The order is determined by the amplitude on the right side in each subject.

Table 5. *The effect of shoulder position on the amplitude of forearm rotation*

(Each value for amplitude is the average of thirty observations)

		Average amplitude (°)		
Subject	Side	Arm adducted	Arm flexed 90°	Difference
1	R.	131	126	+ 4
	L.	143	147	− 2
2	R.	138	131	+ 6
	L.	135	130	+ 5
3	R.	140	150	−10
	L.	136	145	− 9
4	R.	155	153	+ 2
	L.	168	156	+12
Mean		143	142	+ 1

Total rotation (Fig. 6). Although there was a wide range of individual variation (179–292°) of the amplitude of total rotation, variation in the contribution of humeral rotation to total rotation in each case was much less, 90 % of the values falling between 48 and 61 %. In forty-four of the fifty readings, humeral rotation was responsible for more than half of the total movement.

The difference in the amplitude of total rotation with the elbow extended and that of the forearm rotation with the elbow flexed represents that between pronation and supination of the hand in the two positions of the elbow. 90 % of the values with the elbow flexed were between 53 and 69 % of that with the elbow extended.

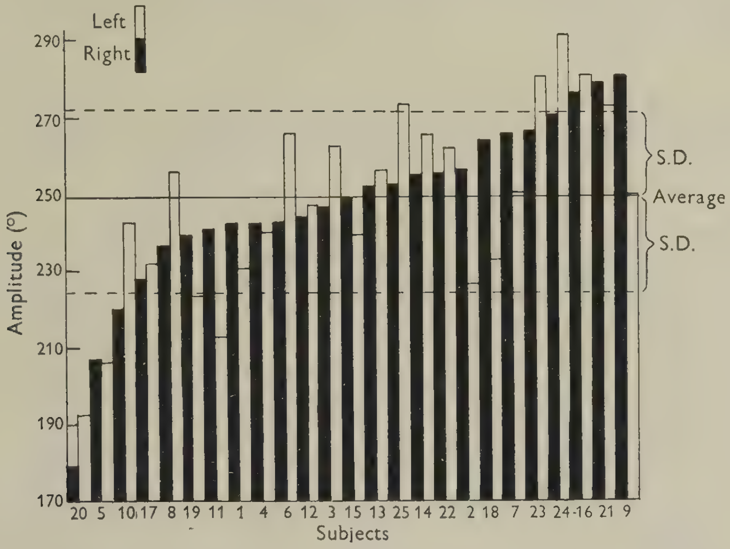

Fig. 6. Histogram of the average amplitude of total rotation for each subject on the right and left sides. The average and standard deviation for all subjects is indicated. The order is determined by the amplitude on the right side in each subject.

Table 6. *The effect of age on the amplitudes of rotation*

(Comparison between the results from two groups of 10 male subjects, with age ranges 16–23 years (*A*) and 29–38 years (*B*))

		Amplitude (°)			
		A		*B*	
Movement measured	Side	Av.	S.D.	Av.	S.D.
Forearm rotation					
Elbow flexed	R.	152	10·5	144	17·3
	L.	154	8·6	147	17·4
Elbow extended	R.	116	15·6	108	15·9
	L.	109	15·3	109	15·2
Humeral rotation					
Elbow flexed	R.	136	11·3	140	6·2
	L.	141	11·6	146	14·0
Elbow extended	R.	128	14·2	135	18·6
	L.	134	9·0	130	17·1
Total rotation	R.	244	21·3	243	26·2
	L.	243	18·1	238	26·0

The effect of age (Table 6). Of the twenty male subjects used in the experiment, ten were between 16 and 23 years of age (average 19·5) and ten between 29 and 38 years of age (average 31·2). On comparison of the values for the various movements in the two groups, no significant differences were found.

The effect of sex (Table 7). A comparison between the amplitudes recorded in the group of five female subjects between the ages of 17 and 28 years (average 22·4) and in the group of eleven male subjects in a similar age group—16–29 years (average 20·4)—showed that in every movement the average for the females exceeded that for the males. In total rotation and humeral rotation with the elbow extended these differences reached a level of statistical significance.

Table 7. *The effect of sex on the amplitudes of rotation*

(Comparison between the results from a group of 11 male subjects ages 16–29 (A) and a group of 5 female subjects ages 17–28 years (B))

		Amplitude (°)			
		A		*B*	
Movement measured	Side	Av.	S.D.	Av.	S.D.
Forearm rotation					
Elbow flexed	R.	149	14·5	157	13·1
	L.	153	8·8	161	8·7
Elbow extended	R.	115	15·1	123	14·3
	L.	106	16·8	129	16·2
Humeral rotation					
Elbow flexed	R.	136	10·7	143	18·2
	L.	142	11·5	146	15·8
Elbow extended	R.	129	13·6	142	11·7
	L.	134	8·6	148	15·8
Total rotation	R.	244	20·2	265	11·0
	L.	240	19·5	276	10·8

The amplitude on the right and left sides (Table 4, Figs. 4–6). The degree and direction of the differences between the amplitude recorded on the two sides varied from movement to movement and from subject to subject. Taking all the results together, there was no significant difference between the two sides, although in seventy-two of the hundred groups of direct readings the differences were statistically significant. However, in only two instances were the differences more than 20%, whereas eighty were less than 10%. A positive rank correlation was found in all groups of direct readings except that of humeral rotation with the elbow extended; that is, except in this case, the values from the two sides were of the same order. In humeral rotation with the elbow extended the largest variations were found—eight being above 10%.

In any one individual, the differences were not necessarily in the same direction for different movements. In fact, this occurred in only four of the twenty-five subjects. In thirteen subjects the differences in three of the four movements measured directly were in the same direction. Fifty-five of the readings were greater on the left side and forty-four on the right, and in one case it was the same on both sides.

The amplitude of rotation from a neutral point (Table 8). The relative proportion of the medial and lateral components of the total amplitude is not consistent from subject to subject nor from the right to the left side in the same subject, so that the value of one cannot be predicted from the other. This is indicated by the lack of a positive rank correlation between the two and by the scatter of the position of the mid-points of the amplitudes from the neutral point. This lack of correlation is also reflected in the fact that the sum of the ranges of values for the medial and lateral components greatly exceeds that for the total movement.

Table 8. *The amplitudes of rotations analysed into medial and lateral components from a neutral point, with the mid-points of the total amplitudes defined in relation to this point*

Neutral point for forearm and total rotation with the distal ends of the radius and ulna in the same vertical plane and for humeral rotation with the forearm vertical. Medial rotation is expressed as a minus value and lateral rotation as a plus value. Humeral rotation and forearm rotation with the elbow extended were not analysed owing to the difficulty of defining a neutral point when using the elbow-cuff arthrometer.

	Amplitude (°)			
	Right		Left	
	Av.	S.D.	Av.	S.D.
Forearm rotation with elbow flexed				
Medial	− 70	11·1	− 74	10·8
Lateral	+ 80	10·3	+ 79	10·9
Mid-point	+ 5	7·8	+ 3	8·3
Humeral rotation with elbow flexed				
Medial	−116	8·7	−118	9·6
Lateral	+ 23	9·2	+ 26	9·2
Mid-point	− 46	7·5	− 46	8·7
Total Rotation				
Medial	−170	15·8	−174	16·9
Lateral	+ 78	15·4	+ 74	15·2
Mid-point	− 46	10·0	− 50	10·0

DISCUSSION

Comparison with previous results

The only results with which those of the present investigation can be compared were obtained in the previous study of the amplitude of forearm rotation (Darcus & Salter, 1953) and by Glanville & Kreezer (1937) on the amplitude of humeral rotation. The figures for the amplitude of forearm rotation with the elbow flexed agree quite closely with those previously obtained with the wrist-cuff arthrometer. The average values given for medial and lateral rotation of the humerus given by Glanville & Kreezer are greater by 25 and 13° respectively and, although using a smaller group of subjects, their range of individual variation is greater. Part at least of these differences may possibly be accounted for by the different methods used.

The amplitude of forearm rotation with the elbow flexed and extended

The finding that the amplitude of forearm rotation was in all subjects significantly greater with the elbow flexed than extended, may possibly be explained by the increase in the distances between the origins and insertions of certain of the antagonist muscles when the elbow is in the extended position. In this position these muscles may be stretched so that a reflex contraction is elicited earlier in the movement.

Because of this difference in forearm rotation in the two positions of the elbow, the amplitude of humeral rotation with the elbow extended cannot be derived by determining the difference between pronation and supination with the elbow extended and flexed. This procedure will give an underestimate of the amplitude.

The amplitude of humeral rotation with the elbow flexed and extended

The amplitude of humeral rotation is not affected consistently by the position of the elbow. If muscles are the limiting factor, it might be anticipated that there would be no significant difference, as the relations between the origins and insertions of the lateral and medial rotators of the humerus are not affected by the position of the elbow. However, that this may not be the main factor, at least in this case, is indicated by the fact that in some individuals the amplitude is greater with the elbow flexed and in others with the elbow extended.

Individual variations

One of the interesting features of this study was the wide range of variation that exists between different individuals. These variations may be related to age or sex.

The effect of age. The progressive effect of 'wear and tear' are common in articular structures with advancing years (Todd, 1939; Bennett, Waine & Bauer, 1942), and it might be expected that these age changes would be reflected in a reduction in the amplitude through which the joint can be moved. However, in the present study, as in the previous one (Darcus & Salter, 1953), no correlation could be demonstrated between age and the amplitude of movement. This may be explained by the fact that the age group studied was restricted; all but four of the twenty-five subjects being under the age of 30. It is of some interest in this respect to note that the oldest subject (aged 38) gave some of the smallest amplitudes recorded, and had older subjects been studied, an age change might perhaps have become apparent. The only other references to the effect of age on joint movement are those of Hewitt (1928) and West (1945). Hewitt found no significant difference in the amplitude of wrist movement in her 112 female subjects whose ages ranged from 11 to 45 years. West noted a slight but progressive limitation (about 10% in all) of the normal amplitude of flexion and extension of the shoulder and of the wrist between the age groups 0–9 years and 70–79 years.

The effect of sex. A comparison of the raw data obtained in the group of female subjects and a group of male subjects of similar age suggested that the amplitude of movement is greater in the female than in the male. However, three of the five differences noted were not statistically significant. Cobe (1928) found insignificant sex differences in the movement of the wrist. On the other hand, Sinelnikoff & Grigorowitsch (1931) were so impressed with the greater amplitude of movement of various joints of their female subjects that they believed that the difference could be considered as a secondary sex characteristic.

A comparison between the amplitudes of movements on the right and left sides

That no marked difference was found between the average values for the amplitude of movement on the right and left sides confirmed the results of Glanville & Kreezer (1937) and the assumption of Patrick (1946) on the amplitude of pronation and supination. Cobe (1928) and Hewitt (1928) found no significant difference in the movements of the wrist joint on the two sides, and Gilliland (1921), in compiling his tables of 'normal' amplitudes of joint movement, stated that there was so little variation between the two sides that they could be presented as combined values.

Although no large differences were found when considering the results as a whole, in some individuals there were differences between the measurements taken on the right and left sides which reached a significant level. However, for clinical purposes, the assumption that the amplitude of movement of a particular joint is the same on the two sides is probably accurate enough, especially if several series of comparative readings are taken. Indeed, since individual variation is so large, unless one has been fortunate enough to measure the amplitude of movement of the joint before injury the amplitude of movement of the opposite joint forms a much more reliable standard of comparison than an average value for the same joint in another individual.

SUMMARY

1. An investigation is described of the amplitude of forearm rotation and humeral rotation with the elbow flexed and of total or combined forearm and humeral rotation with the elbow extended.

2. Forearm rotation with the elbow flexed was consistently larger than with the elbow extended. In the majority of instances the difference exceeded 15 %. This finding may be explained by alteration in distances between the origin and insertion of muscles participating in the movement in the two positions of the elbow.

3. No consistent effect was found of elbow position on humeral rotation. In general, in the flexed position of the elbow, humeral rotation was larger, but in the majority of instances the difference was less than 10 %.

4. Humeral rotation in general contributed more to total rotation with the elbow extended than did forearm rotation. The average was 54 %, with 90 % of the values falling between 48 and 61 %.

5. No effect of age on the amplitude was found in the groups studied, the average ages of which were 19·5 and 31 years.

6. The average amplitudes recorded from the female subjects were in all cases greater than in the males of a similar age group, but these were only statistically significant in total rotation and humeral rotation with the elbow extended.

7. The amplitudes on the right and left sides tended to be of the same order, although statistically significant differences were found. For practical purposes it is concluded that, in spite of these differences, the amplitude recorded from one limb is the most reliable standard of normality or otherwise of the amplitude of the corresponding movement on the other side.

REFERENCES

Appleton, A. B., Hamilton, W. J. & Simon, G. (1948). *Surface and Radiological Anatomy*, 2nd. ed., pp. 46–70. Cambridge: Heffer and Sons.

Bennett, G. A., Waine, H. & Bauer, W. (1942). *Changes in the Knee Joint at Various Ages.* New York: Commonwealth Fund.

Brash, J. C. & Jamieson, E. B. (1943). *Cunningham's Textbook of Anatomy*, 8th ed., p. 353. London: Humphrey Milford.

Bryce, T. H. (1915). *Quain's Elements of Anatomy*, 11th ed., vol. 4, pt. 1, p. 240. London: Longmans, Green and Co.

Clark, W. A. (1920). A system of joint measurements. *J. orthop. Surg.* 2, 687–700.

Cobe, H. M. (1928). The range of active motion at the wrist of white adults. *J. Bone Jt. Surg.* **10**, 763–774.

Darcus, H. D. (1951). The maximum torques developed in pronation and supination of the right hand. *J. Anat., Lond.*, **85**, 55–67.

Darcus, H. D. & Salter, Nancy (1953). The amplitude of pronation and supination with the elbow flexed to a right angle. *J. Anat., Lond.*, **87**, 169–184.

Dorinson, S. M. & Wagner, M. L. (1948). An exact technic for clinically measuring and recording joint motion. *Arch. phys. Med.* **29**, 468–475.

Frescoln, L. D. (1929). Range of bodily movements. *Med. Times, N.Y.*, **57**, 197–198.

Gilliland, A. R. (1921). Norms for amplitude of voluntary movement. *J. Amer. med. Ass.* **77**, 1357.

Glanville, A. D. & Kreezer, G. (1937). The maximum amplitude and velocity of joint movements in normal male human adults. *Human Biol.* **9**, 197–211.

Hewitt, D. (1928). Range of active motion at the wrist of women. *J. Bone Jt. Surg.* **10**, 775–787.

Johnston, T. B. & Whillis, J. (1946). *Gray's Anatomy*, 29th ed., pp. 476–484. London: Longmans, Green and Co.

Macalister, A. (1889). *A Textbook of Human Anatomy*, p. 146. London: Griffin and Co.

Mainland, D. (1947). *Anatomy as a Basis for Medical and Dental Practice*, p. 226. London: Hamish Hamilton.

Patrick, J. (1946). A study of supination and pronation, with especial reference to the treatment of forearm fractures. *J. Bone Jt. Surg.* **28**, 737–748.

Rosen, N. G. (1922). A simplified method of measuring amplitude of motion in joints. *J. Bone Jt. Surg.* **4**, 570–579.

Silver, D. (1923). Measurement of the range of motion in joints. *J. Bone Jt. Surg.* **5**, 569–578.

Sinelnikoff, E. & Grigorowitsch, M. (1931). Die Beweglichkeit der Gelenke als sekundäres geschlechtliches und konstitutionelles Merkmal. *Z. ges. Anat.* 2. *Z. KonstLehre*, **15**, 679–693.

Todd, T. W. (1939). Skeleton, locomotor system and teeth. In *Problems of Ageing*, ed. E. V. Cowdrey, pp. 278–338. London: Baillière, Tindall and Cox.

West, C. C. (1945). Measurement of joint motion. *Arch. phys. Med.* **26**, 414–425.

CELLULAR PROLIFERATION IN THE PROXIMAL SEGMENT OF A SECTIONED OR CRUSHED PERIPHERAL NERVE

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INTRODUCTION

The concentration of total nucleic acid increases in the distal segment of a sectioned or crushed peripheral nerve, reaching a maximum at 16 days (Logan, Mannell & Rossiter, 1952*a*). The change involves pentosenucleic acid (PNA) and deoxypentosenucleic acid (DNA), although the increase in the two nucleic acids is disproportionate, resulting in an increase in the PNA/DNA ratio. The concentration of nucleic acid in the distal segment falls after 16 days, although the normal value is not yet restored 600 days after damage to the nerve. These chemical changes are explained by the well-known cellular proliferation which occurs during Wallerian degeneration. Abercrombie & Johnson (1946) placed the changes in cell population during Wallerian degeneration on a quantitative basis by estimating the number of nuclei in a unit volume of the affected nerve.

In a study of the chemical changes in the proximal segment of a damaged nerve, Logan (1952) found quite large increases in the concentrations of both PNA and DNA after either nerve section or nerve crush. These increases in the concentration of nucleic acids, which are not so marked as those in the distal segment, suggest that some cellular proliferation occurs, not only in the distal segment, but also in the proximal segment following either nerve section or nerve crush. Compared with Wallerian degeneration in the distal segment, the histological basis for the observed changes in nucleic acids in the proximal segment is not well established. Most histologists have felt that the traumatic degeneration of the nerve fibres extends proximally for very short distances, probably not past the first or second node of Ranvier (Ramón y Cajal, 1928). However, Ranson (1912) stated that the degenerative changes were limited to 2 cm. in the sciatic nerve of the dog, and Mönckeberg & Bethe (1899) reported degenerative changes as high as 3–4 cm. from the point of section for the sciatic nerve of the rabbit. In view of these divergent observations and the emphasis, in histological studies, on the degenerative changes in the axis cylinders and myelin sheaths, a search was made for signs of cellular proliferation in the proximal segment, in order to explain the increases in nucleic acids which were observed in chemical studies.

MATERIAL AND METHODS

In five mature cats, a 1·5 cm. length of the right sciatic nerve was resected midway between the greater trochanter of the femur and the division of the greater sciatic nerve into its tibial and common peroneal branches. In a second series of five mature cats, the right sciatic nerve was crushed with a haemostat at the same level. The operations were done with full aseptic precautions under nembutal anaesthesia.

Survival times were 16 days for two animals and 96 days for three animals, in each series.

Blocks were removed as follows, in order to make nuclear counts according to method I of Abercrombie (1946). A 1 cm. length of nerve adjacent to the point of section or crush was rejected for both the proximal and distal segments. This was the procedure adopted in the chemical studies of Logan (1952). A 5 cm. length of nerve was removed from the remainder of the proximal and distal segments and divided into three blocks after fixation according to Susa. Blocks A and B of each segment were 1·0–2·7 cm. and 4·3–6·0 cm. from the site of section of crush, respectively. Nuclear counts were done for blocks A and B. The intervening portion of nerve (2·7–4·3 cm. from the site of injury) was used for measuring nuclear lengths, as required for method I of Abercrombie. Similar lengths of the left sciatic nerve from the corresponding region were removed to serve as controls.

The various blocks were embedded in paraffin and sectioned at $7\,\mu$. Blocks A and B were sectioned transversely; the intervening portion of nerve was sectioned longitudinally. The sections were stained with iron haematoxylin and counterstained with a 1 % solution of Orange G. The method of estimating the number of nuclei per unit volume of nerve is described in detail by Abercrombie (1946). For our present purpose, no attempt was made to identify the various cell types on the basis of nuclear morphology.

RESULTS

Table 1 shows the mean percentage increase in the nuclear population in block A (1·0–2·7 cm. from site of injury) and block B (4·3–6·0 cm. from site of injury) for the proximal and distal segments. Since there were no significant differences between the findings for the cut and the crushed nerves, the data have been pooled for statistical treatment.

Table 1. *Mean percentage increase in the nuclear population of the sciatic nerve of the cat after either nerve section or nerve crush*

Time after operation (days)	Distance from site of injury (cm.)	No animals	Percentage change (mean ± S.E.M.) Proximal segment	Percentage change (mean ± S.E.M.) Distal segment
16	1·0–2·7 (block A)	4	172 ± 64	518 ± 46
96	1·0–2·7 (block A)	6	179 ± 50	565 ± 28
16	4·3–6·0 (block B)	4	4·7 ± 2·8	435 ± 27
96	4·3–6·0 (block B)	6	52 ± 13	540 ± 78

In block A, 1·0–2·7 cm. from the site of injury, there was a significant increase in the cellularity of the proximal segment both 16 and 96 days after the operation. The difference between the increase for the two time periods was not significant statistically ($P > 0{\cdot}9$). In block B, 4·3–6·0 cm. from the site of injury, the increase in cellularity 16 days after the operation was not significant statistically, but at

96 days the increase was definite, and it was significantly greater ($P < 0{\cdot}02$) than at 16 days. Both at 16 and 96 days the increase at the level nearer the site of injury was significantly greater ($P < 0{\cdot}05$) than at the level farther removed.

At both levels and at both time intervals the increase in cellularity in the distal segment was significantly greater than the corresponding increase in the proximal segment. In the distal segment there was no significant difference between the figures for the two levels at either of the two time intervals, or between the figures for the two time intervals at either of the two levels.

DISCUSSION

The changes in the nuclear population in the distal segment at both 16 and 96 days are in good agreement with the figures reported by Abercrombie & Johnson (1946) for the degenerating sciatic nerve of the rabbit. The finding that in the distal segment there was no significant difference between the mean increase at each of the two levels confirms the finding of Joseph (1948) for the splanchnic nerve of the rabbit.

Any or all of Schwann cells, fibrocytes, macrophages and cells associated with blood vessels may participate in causing an increase in nuclear population in the proximal segment of a cut or crushed peripheral nerve. The cellular proliferation is more marked both in degree and in extent along the nerve than previous studies would suggest. This increase in cell population in the proximal segment almost certainly accounts for the increase in the concentration of nucleic acid reported by Logan (1952), as it does in the distal segment Logan *et al.* (1952*a*). It should be borne in mind, however, that the figures obtained for nuclear population and those obtained for DNA estimations are not strictly comparable. The concentration of DNA depends upon the number of nuclei throughout a considerable length of nerve, while the counting method gives the nuclear population density at particular levels.

Bodian & Dziewiatkowski (1950) reported that the increase in the concentration of 'residual P' in the proximal segment of monkey nerves 7–23 days after nerve section was greater than that in the distal segment, the inference being that there was a greater increase in the concentration of nucleic acid in the proximal than in the distal segment. In this regard it should be remembered that less than one-half of the 'residual P' of a myelinated peripheral nerve is derived from nucleic acid. More than one-half of the protein-bound phosphorus, or 'residual P', is derived from some compound, or group of compounds, possibly neurokeratin, associated with the myelin sheath of the fibre (Logan *et al.* 1952*a* and *b*; Logan, 1952). In the degenerating distal segment, where the myelin sheaths disintegrate, the concentration of this residual organic phosphorus falls as the concentration of nucleic acid increases. In the proximal segment, where there is little myelin sheath destruction, there is no such concomitant fall in the concentration of residual organic phosphorus.

Increase in the cellularity of the proximal segment probably accounts for the increase in acid phosphatase activity found by Bodian (1947) in the proximal segments of monkey nerves 10–14 days after nerve section. Bodian suggested that the increased acid phosphatase activity might be due to some change within the axon. This suggestion would appear to be unlikely, since in the degenerating distal segment there is a good general association between acid phosphatase activity, cellularity and concentration of DNA (Hollinger, Rossiter & Upmalis, 1952).

SUMMARY

1. The changes in total nuclear population were determined at two different levels in both the proximal and distal segments of the sciatic nerve of the cat at 16 and 96 days after either nerve section or nerve crush.

2. In the proximal segment the nuclear population was increased 1·0–2·7 cm. from the site of injury at both 16 and 96 days after the operation. At 4·3–6·0 cm. from the site of injury, there was no increase in nuclear population 16 days after the operation, but by 96 days there was a small, but statistically significant, increase. The increase in nuclear population was greater in the distal segment at both time intervals, compared with the proximal segment.

3. The cellular proliferation in the proximal segment, shown by the increase in nuclear population, explains the increase in nucleic acids (PNA and DNA) which had previously been demonstrated in the sciatic nerve proximal to the site of injury.

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REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–247.

ABERCROMBIE, M. & JOHNSON, M. L. (1946). Quantitative histology of Wallerian degeneration. I. Nuclear population in rabbit sciatic nerve. *J. Anat., Lond.*, **80**, 37–50.

BODIAN, D. (1947). Nucleic acid in nerve-cell regeneration. *Symp. Soc. exp. Biol.* **1**, 163–178.

BODIAN, D. & DZIEWIATKOWSKI, D. (1950). The disposition of radioactive phosphorus in normal, as compared with regenerating and degenerating nervous tissue. *J. cell. comp. Physiol.* **35**, 155–177.

HOLLINGER, D. M., ROSSITER, R. J. & UPMALIS, H. (1952). Chemical studies of peripheral nerve during Wallerian degeneration. 4. Phosphatases. *Biochem. J.* **52**, 652–659.

JOSEPH, J. (1948). Changes in nuclear population following twenty-one days' degeneration in a nerve consisting of small myelinated fibres. *J. Anat., Lond.*, **82**, 146–152.

LOGAN, J. E. (1952). Protein-bound phosphorus compounds in the proximal stump of peripheral nerve after nerve section or after nerve crush. *Canad. J. Med. Sci.* **30**, 457–462.

LOGAN, J. E., MANNELL, W. A. & ROSSITER, R. J. (1952*a*). Chemical studies of peripheral nerve during Wallerian degeneration. 3. Nucleic acids and other protein-bound phosphorus compounds. *Biochem. J.* **51**, 482–487.

LOGAN, J. E., MANNELL, W. A. & ROSSITER, R. J. (1952*b*). Estimation of nucleic acids in tissue from the nervous system. *Biochem. J.* **51**, 470–482.

MÖNCKEBERG, G. & BETHE, A. (1899). Die Degeneration der markhaltigen Nervenfasern der Wirbelthiere unter hauptsächlicher Berücksichtigung des Verhaltens der Primitivfibrillen. *Arch. mikr. Anat.* **54**, 135–183.

RANSON, S. W. (1912). Degeneration and regeneration of nerve fibres. *J. comp. Neurol.* **22**, 487–537.

RAMÓN Y CAJAL, S. (1928). *Degeneration and Regeneration in the Nervous System.* London: Oxford University Press.

THE DEVELOPMENT OF THE RABBIT VAGINA

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Although it is agreed that the upper part of the rabbit's vagina develops from the Mullerian canals, three conflicting views exist concerning the composition of the lower part. Langenbacher (1882) and Mihalkovics (1885) considered that the lower part, like the upper, is composed of Mullerian tissue. Later Baxter (1933) concluded that this lower part is formed from the epithelium of the persistent bases of the Wolffian ducts. Finally, Jost (1943*a*, *b*, 1947) considered that it is derived from tissue of the urogenital sinus, the Wolffian ducts completely disappearing. In view of these discrepant results, it seemed useful to undertake a new description of the development of the rabbit vagina, based on a full series of specimens prepared for histological analysis.

MATERIALS AND METHOD

The foetuses used in this work were grouped according to the age since mating, and not according to size, which is an unreliable index of age (Waterman, 1942; Jost, 1947). Foetuses were obtained at fifteen stages, mostly at daily intervals between 19 and 32 days post-coitum. At least two specimens of each age were examined. They were placed in a warm saline bath, the abdominal wall and pelvic girdle were opened, Bouin's fixative was injected into the bladder and rectum, and the whole foetus was then immersed in Bouin. This method always ensured satisfactory fixation. The reproductive organs were dissected out, embedded in paraffin, sectioned at 10μ and stained in Weigert's haematoxylin and Orange G with Erythrosin. This method of staining was found to emphasize the distinction between the Wolffian and sinus cells. Graphical reconstructions were made of the reproductive ducts using one specimen of each of the fifteen stages. The other specimen was used to check the results obtained from each reconstruction.

RESULTS

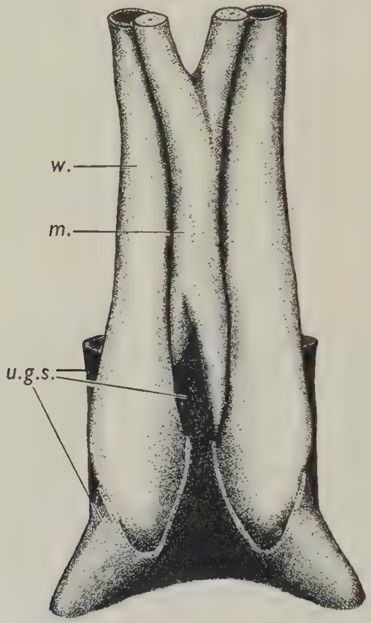
(*a*) *Identification of tissues*

In the early stages the Wolffian cells appear as a columnar epithelium, the cytoplasm of which stains particularly with Orange G, while the nuclei stain lightly with Weigert's haematoxylin. In the later stages the epithelial character is lost but the staining properties remain the same. Because of its more closely packed nuclei, the Mullerian tissue stains much more darkly with haematoxylin than does the Wolffian tissue. The distinction between the two epithelia is shown particularly in Pl. 1, figs. 1 and 2. The urogenital sinus tissue consists of loosely packed irregular cells, the cytoplasm staining with both Orange G and Erythrosin, giving the cells a pinker colour than that of the Wolffian tissue.

(*b*) Description of stages

It should be noted that throughout the description, posteriorly is synonymous with caudally and anteriorly with cephalically.

19- *and* $19\frac{1}{2}$-*day embryo.* At these stages the Mullerian canals open to the peritoneal cavity by the ostia. Each Mullerian canal consists of a thick darkly staining epithelium round a small lumen. As one proceeds caudally the canals approach each other, becoming interposed between the two Wolffian canals, and joining each other for a short distance to form the rudiments of the Mullerian vagina (Text-fig. 1).



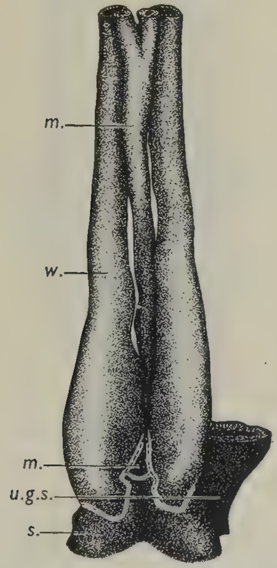
Text-fig. 1. Dorsal reconstruction from $19\frac{1}{2}$-day female embryo. × 100. *w*, Wolffian tissue; *m*, Mullerian tissue; *s*., sinus tissue projecting from the dorsal wall of the sinus; *u.g.s.*, urogenital sinus proper; *p*., projection from the Mullerian vagina. (These abbreviations apply to all figures.)

At 19 days the lumina of the two canals are still distinct though their walls are fused medially. At $19\frac{1}{2}$ days a complete fusion of the lumina occurs for a short distance. In both stages the two canals diverge posteriorly, each tapering away to nothing on the wall of the Wolffian canal of its own side, and ending before the sinus is reached.

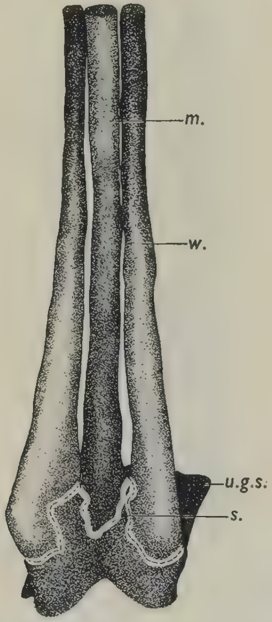
The Wolffian ducts are large with a thinner and more lightly staining epithelium than that of the Mullerian canals. They are continuous from the mesonephros to the urogenital sinus. The lumina are large and considerably swollen just anterior to their communication with the sinus. They open into two projections from the dorsal wall of the urogenital sinus which in turn open into the urogenital sinus proper.

21-*day embryo.* The Mullerian ducts have joined more extensively to form the vagina, and posteriorly the Wolffian canals become apposed to its lateral walls. The divergent posterior ends of the Mullerian canals are still present as in the previous stages. The solid base of the Mullerian vagina divides into right and left arms adjacent to the Wolffian bulbs where these open into the two projections from the dorsal wall of the sinus.

22-*day embryo.* The Mullerian canals are joined more extensively and are separated no longer at their extreme posterior tips into right and left parts as in the previous stages (Text-fig. 2). The Mullerian vagina terminates in a dorsal sheet of tissue inserted between the Wolffian canals and bounded posteriorly by sinus tissue. The large expansion of the posterior part of the Wolffian canals almost obscures the Mullerian vagina when viewed from the dorsal side. The Wolffian canals still open into the urogenital sinus.



Text-fig. 2. Dorsal reconstruction from 22-day female embryo. × 50.



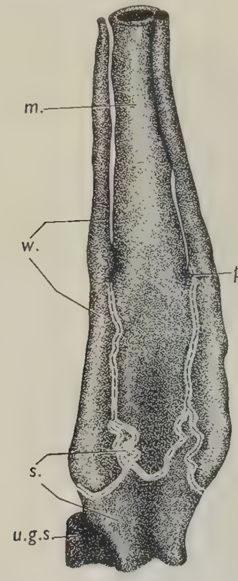
Text-fig. 3. Dorsal reconstruction from 24-day female embryo. × 50.

23-*day embryo.* The Mullerian vagina is like that of the previous stage but posteriorly the solid tip has become separated by the urogenital sinus into dorsal and ventral arms. The dorsal arm is embedded in the sinus tissue for some distance. The anterior parts of the Wolffian canals have completely degenerated while posteriorly their openings into the urogenital sinus are blocked by loosely packed sinus cells. Thus the two projections from the dorsal wall of the sinus fuse to form a solid core in which the ends of the Mullerian canals terminate.

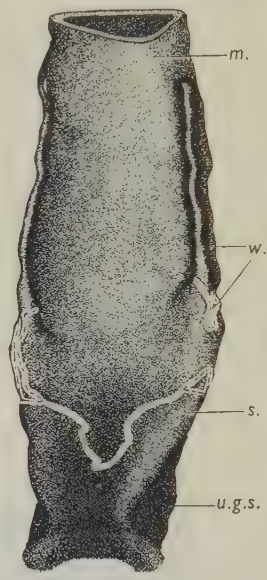
24-*day embryo.* The Mullerian vagina has increased in size while the Wolffian canals have undergone further degeneration. The projection from the dorsal wall of the sinus ((*s.*) in Text-fig. 3) has grown anteriorly and forms a considerable structure joining the Mullerian and Wolffian canals to the urogenital sinus.

25-*day embryo.* Further growth of the Mullerian vagina and of the projections from the dorsal wall of the urogenital sinus is accompanied by further degeneration of the Wolffian canals. Dorso-lateral projections (*p.* in Text-fig. 4) have grown out from the Mullerian vagina to meet the Wolffian ducts. Posteriorly from here an extensive fusion of the Wolffian canals to the lateral walls of the Mullerian vagina has occurred; although in the most posterior part they are separated from the Mullerian by the sinus tissue.

26-*day embryo*. The Mullerian projections are more extensive while there is further degeneration of Wolffian tissue at this age compared with that of the 25-day embryo. The left Wolffian duct still remains swollen at its base, where there is a small lumen, whereas the right is reduced to a solid rod of cells.



Text-fig. 4. Dorsal reconstruction from 25-day female embryo. ×50.



Text-fig. 5. Dorsal reconstruction from 27½-day female embryo. ×33.

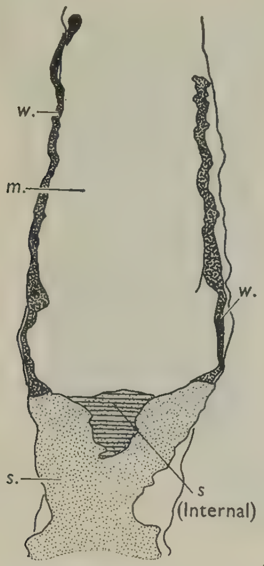
27-*day embryo*. The Wolffian canals have undergone such a degree of degeneration that nowhere is there a lumen present. The right Wolffian duct has fragmented. Both ducts at this stage have been reduced to a single cell in thickness in many parts, and only by careful tracing from section to section is it possible to follow them. Posteriorly they are joined to the vagina by the two lateral projections of Mullerian tissue, and they become embedded in the wall of the vagina meeting the anteriorly projecting sinus tissue.

At this stage the sinus tissue is not only embedded in the solid tip of the Mullerian vagina, dividing it into dorsal and ventral arms, as in the previous stages, but also projects anteriorly into the lumen, forming a solid core of sinus tissue. Hence the posterior end of the vagina is composed of this solid core of tissue, which extends a short way along the sides while the Mullerian tissue forms the dorsal and ventral walls.

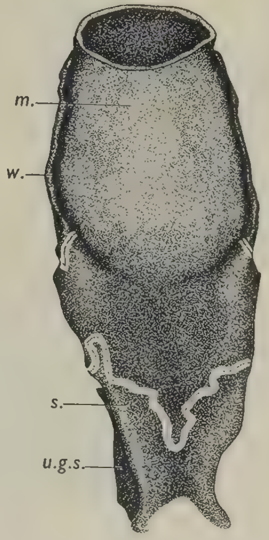
27½-*day embryo*. The Wolffian tissue is in a similar degenerate condition to that of the previous stage (Text-fig. 5). Posteriorly the right Wolffian duct passes to the inside of the Mullerian epithelium and emerges again to join the sinus tissue.

Text-fig. 6 is the reconstruction of the dorsal view showing also the internal distribution of tissues. The internal Wolffian and sinus tissue is seen here in relation to the other tissues.

The degenerate condition of the Wolffian tissue and its incorporation beneath the Mullerian tissue on the right side is seen in Pl. 1, fig. 3. This section also shows the projection of the Mullerian tissue which has extended from the vagina to incorporate the Wolffian ducts.



Text-fig. 6. As Text-fig. 5, but to show general distribution of tissues. × 33.



Text-fig. 7. Dorsal reconstruction from 28½-day female embryo. × 33.

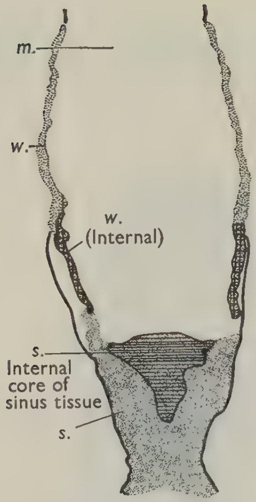
28½-*day embryo.* At this stage (Text-fig. 7) the bases of the Wolffian canals have become completely incorporated internally to the Mullerian vaginal epithelium (Text-fig. 8). On the right side, the Wolffian duct is no longer in contact with the sinus tissue. Further degeneration in the anterior region of the Wolffian canals has taken place.

29-*day embryo.* A great reduction in the Wolffian tissue has taken place at this age (Text-fig. 9). Anteriorly, the Wolffian canals are reduced to a small vestige on the tip of the projections from the Mullerian vagina. Internally, the Wolffian canals have become fragmented and lie below the Mullerian epithelium (Text-fig. 10).

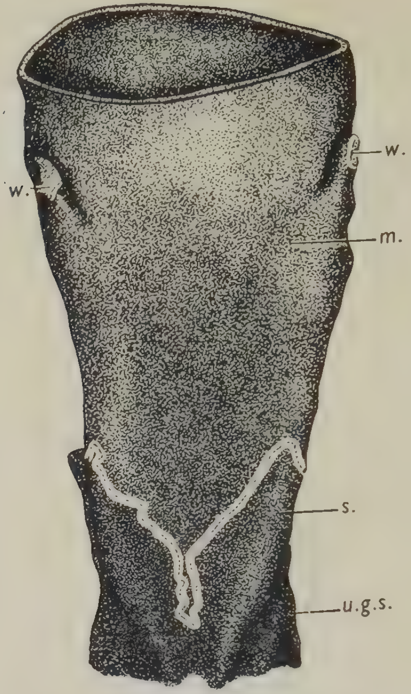
The anterior projections of the sinus tissue which were joined to the posterior ends of the Wolffian tissue have become internal to the Mullerian epithelium. Pl. 1, fig. 4, shows the great reduction in the Wolffian tissue on the right side and its position internally to the Mullerian epithelium.

30-*day embryo.* Only a very small vestige of the Wolffian tissue now remains externally on the tips of the projections from Mullerian vagina. Internally, the Wolffian remnants are even further reduced compared with the previous stage.

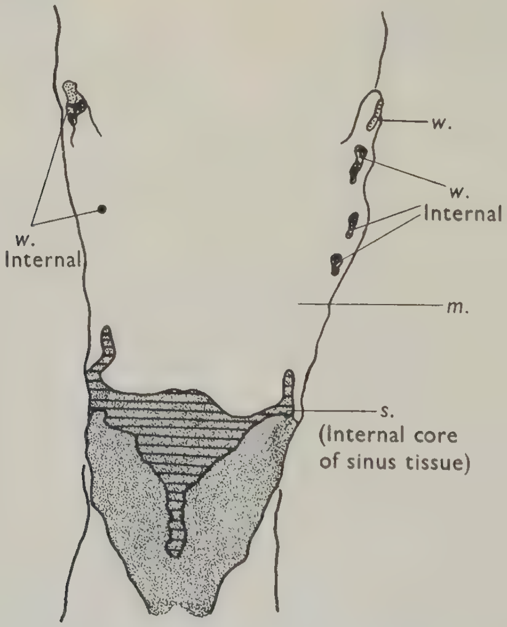
31-*day embryo.* The Wolffian tissue has now completely degenerated externally on the left side while on the right side only a remnant was found embedded in the



Text-fig. 8. As Text-fig. 7, but to show general distribution of tissues. × 33.



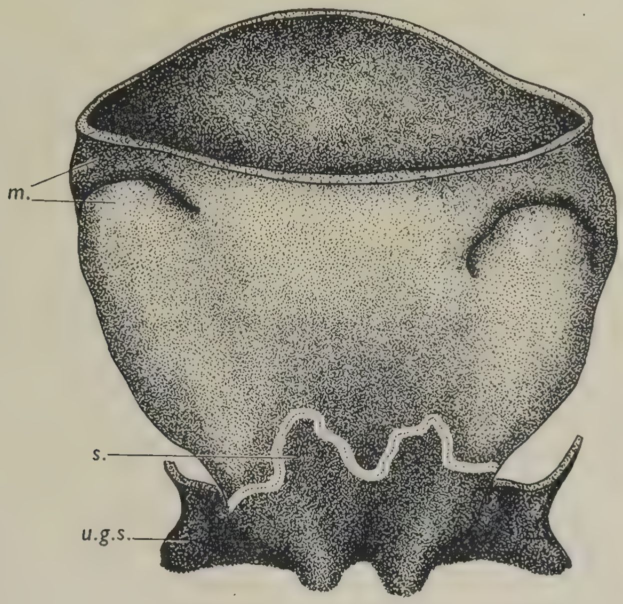
Text-fig. 9. Dorsal reconstruction from 29-day female embryo. × 50.



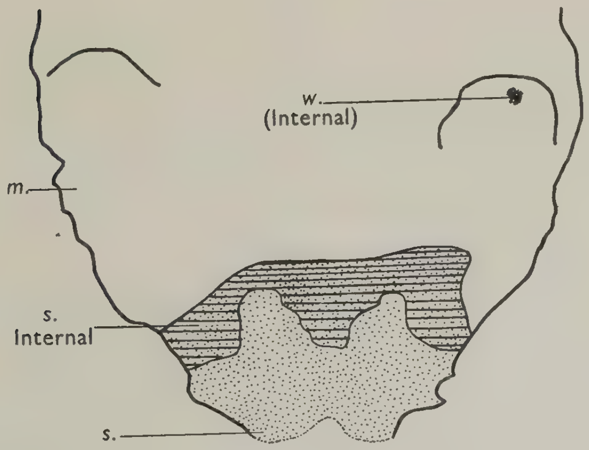
Text-fig. 10. As Text-fig. 9, but to show general distribution of tissues. × 50.

wall of the projection of the Mullerian vagina. Internally, a small fragment was found beneath the epithelium of the vagina of the right side. The sinus tissue forms a solid base to the vagina joining it to the urogenital sinus.

32-*day embryo*. Only the right Wolffian canal was found internally to the Mullerian epithelium, as a small group of cells only 40μ in length (Text-figs. 11 and 12). The sinus tissue forming the base of the vagina is still not canalized.



Text-fig. 11. Dorsal reconstruction from 32-day female embryo. ×50.



Text-fig. 12. As Text-fig. 11 but to show general distribution of tissues. ×50.

DISCUSSION

Histological differentiation between the epithelia of the Mullerian ducts, Wolffian ducts and uro-genital sinus is very clear in these specimens. The description of most stages shows that the limits between the different types of epithelia do not always correspond with the obvious morphological limits. For instance, sinus epithelium extends dorsally and anteriorly from the urogenital sinus to form the terminal part of the morphologically continuous vagina; and the Mullerian vagina forms temporary diverticula which join the degenerating Wolffian canals to it in the later stages. In view of the complete sequence of events in development described here the most tenable explanation is that the histological appearance of the epithelium indicates its origin, and that actual outgrowths of sinus or Mullerian epithelium are responsible for the arrangement observed. The alternative assumption, that a transformation of one type of epithelium into another occurs, seems very unlikely in view of the absence of transitional stages.

The changing position of the borders between epithelial types and the lack of consistent correspondence between morphological and histological boundaries is perhaps responsible for the discrepant opinions about the development of the vagina which have been held by earlier workers (Langenbacher, Mihalkovics & Baxter). The results obtained in the present work confirm the view held by Jost (1943*a*, *b*, 1947) that the vagina of the full-term rabbit foetus is derived mainly from Mullerian tissue, with sinus tissue forming its posterior part. Carleton (1931) found corresponding evidence in the adult for its composite nature, in his study in epithelial phagocytosis. He found that the lower third of the vagina was distinctly different from the upper two-thirds, being columnar and non-stratified. Correspondingly, when Jost (1942) injected testosterone propionate, and Courrier & Jost (1942) injected pregneninolone into pregnant rabbits, they found stimulation of only the lower part of the foetal vagina.

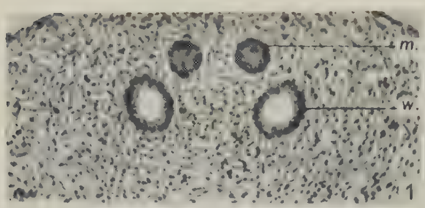
SUMMARY

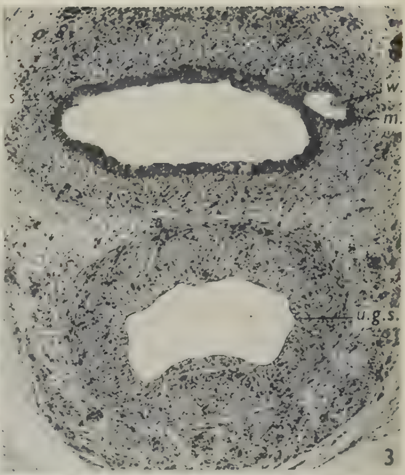
1. The development of the rabbit vagina is described in detail from reconstructions made from serial sections of foetuses of 19–32 days gestation.

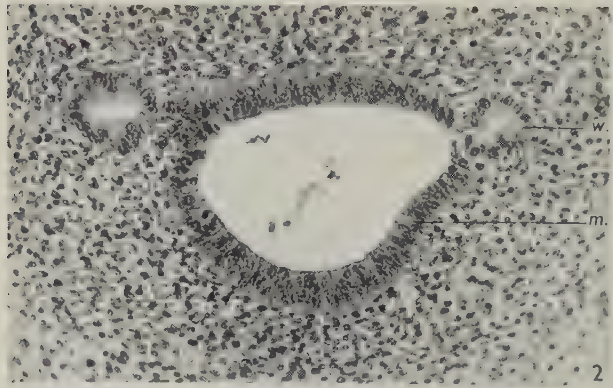
2. The Mullerian ducts join to form the anterior part of the vagina. Two dorsolateral diverticula were formed from Mullerian tissue at 25 days and incorporated the bases of the degenerating Wolffian canals. These diverticula were still present at 32 days. It was found that the Wolffian ducts degenerate almost completely leaving only a small group of cells embedded in the epithelium of the right Mullerian diverticulum. A projection from the dorsal wall of the sinus elongates during development and forms the posterior part of the vagina.

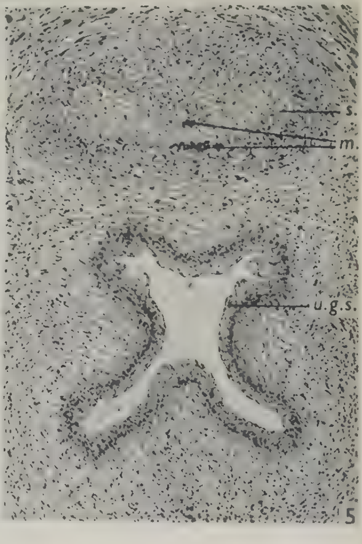
3. These results support the view of Jost that the vagina of the rabbit is formed from Mullerian and sinus tissue but provide no evidence in support of the view of Baxter that it is formed from the Mullerian and Wolffian tissues or of Mihalkovics and Langenbacher that it is formed from Mullerian tissue alone.

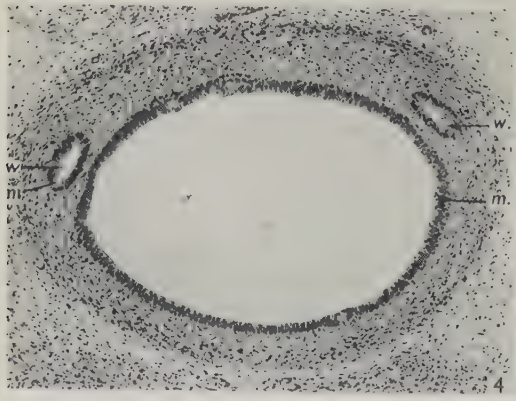
I am indebted to Prof. G. R. de Beer for his help in the initial stages, and subsequently to his successor Mr M. Abercrombie for all his valuable help and advice in the completion and presentation of this work.











CARR—The development of the rabbit vagina

I especially wish to thank Mr H. Barker for his technical advice and skill, especially in the cutting of serial sections; and Miss J. Hubbard for the photographs.

REFERENCES

BAXTER, J. S. (1933). The development of the vagina of the rabbit. *J. Anat., Lond.*, **67**, 555–562.

CARLETON, H. M. (1931). Studies on epithelial phagocytosis. I. *Proc. Roy. Soc.* B, **108**, 1–10.

COURRIER, R. & JOST, A. (1942). Intersexualité foêtale provoquée par la prégnéninolone au cours de la grossesse. *C.R. Soc. Biol., Paris*, **136**, 395–396.

JOST, A. (1942). Intersexualité expérimentale chez l'embryon de lapin. Étude du vagin et de l'utricule prostatique. *Ann. Endocr., Paris*, **3**, 121–124.

JOST, A. (1943*a*). Étude d'embryologie et de physiologie génitales chez le lapin. *Ann. Endocr., Paris*, **4**, 221–238.

JOST, A. (1943*b*). Sur le vagin de la lapine. *C.R. Soc. Biol., Paris*, **137**, 392–393.

JOST, A. (1947). Recherches sur la différentiation sexuelle de l'embryon de lapin. *Arch. Anat. micr. Morph. exp.* **36**, 151–200, 242–270, 271–315.

LANGENBACHER, L. (1882). Beitrag zur Kenntniss der Wolff'schen und Müller'schen Gange bei Säugern. *Arch. mikr. Anat.* **20**, 92–108.

MIHALKOVICS, VON G. (1885). Untersuchungen über die Entwickelung des Harn- und Geschlechtsapparates der Amnioten. *Int. Mschr. Anat. Histol.* **2**, 41–62, 65–106, 284–306, 307–339, 348–385, 387–433, 435–485.

WATERMAN, A. J. (1942). Variation in rabbit embryos. *Anat. Rec.* **84** (Abstract), 513–514.

EXPLANATION OF PLATE

The photomicrographs of sections are shown in anterior view so that the actual left side of the body is the right side of the illustration.

Fig. 1. Transverse section showing the comparative sizes and appearances of the Wolffian and Mullerian canals in a 21-day foetus. × 115.

Fig. 2. Transverse section to show the degenerating Wolffian ducts adhering to the lateral walls of the Mullerian vagina in a 25-day foetus. × 180.

Fig. 3. Transverse section showing the remains of the Wolffian tissue being incorporated in the wall of the Mullerian vagina in a 27½-day foetus. On the right is shown the Mullerian projection which joins the Wolffian duct to the vagina. × 50.

Fig. 4. Transverse section of the 29-day foetus. The Wolffian tissue appear as a small group of cells in the wall of the projections from the Mullerian vagina. × 65.

Fig. 5. Transverse section of the base of the vagina and the urogenital sinus, just anterior to their junction. The tip of the Mullerian vagina is seen connected to the sinus region. × 65.

REVIEWS

The Anatomy of the Nervous System. By S. W. Ranson. 9th edition, revised by S. L. Clark. (Pp. xii + 581; 434 figures. Price 42*s.* 6*d.*) Philadelphia and London: W. B. Saunders and Co. 1953.

This new edition of Ranson's *Anatomy of the Nervous System* differs only slightly from the preceding 8th edition. It is somewhat increased in size, containing fifty more pages and about twenty more figures. The general arrangement and content remains the same, though there has been some re-arrangement within chapters, and many small additions or modifications have been made. A few sections, such as those dealing with the cerebral cortex and the cerebellum, have been more extensively rewritten, but in general the changes which have been made are not of outstanding importance. The book remains a useful and reliable account of the structure of the nervous system, in which much recent work is at least noted.

In addition to dealing with adult structure, this book claims also to deal with both development and function in the nervous system. To cover so wide a field within the limits of a student's text-book is obviously difficult. In fact, adult structure is described in very considerable detail, in greater detail perhaps than is necessary for the medical student, and certainly far more is given than most ever assimilate. Development, on the other hand, is dealt with very briefly, and from a purely morphological point of view. Questions of function are discussed more fully and hardly any structural features are described without some reference to their functional significance. Owing partly to limitations of space, and to the enormous bulk of physiological and clinical publication, much of the functional discussion consists of synoptic lists of experimental and clinical findings, and this is no doubt inevitable. In some cases, however, functional ideas are expressed in such vague terms as to convey very little precise meaning to the reader, and one feels that in many parts of the book, a more critical evaluation of functional interpretations would have been an advantage. For example, the 'rhinencephalon' is described in considerable structural detail and illustrated by the usual complicated diagrams of connexions and fibre tracts; not all the structural features described are really firmly established, and the general attribution of olfactory function to such parts as the hippocampus, mamillary bodies and anterior nuclei of the thalamus is open to considerable doubt, particularly in the light of recent work.

It is of course not surprising that defects of this kind should exist in a text-book which must remain of moderate size and price, and deal with so extensive a subject. Practically any book of a similar kind would be open to the same sort of criticism, and in performing the difficult task of selection and compression it is impossible to please everyone. The task is the more difficult when it becomes necessary to intercalate new material in a book the general form and content of which is already established. One may doubt whether the attempt to keep a book in publication through nine editions, when it deals with a field of work which is advancing and changing so rapidly, can ever be satisfactory without almost complete re-writing, so as to produce substantially a new work.

F. GOLDBY

Second Annual Report on Stress. By Hans Seyle and Alexander Horava. (Pp. viii + 526; $9\frac{1}{2} \times 6\frac{1}{2}$ in.; 16 plates; \$10.00.) Montreal: Acta Inc. 1952.

The present volume is essentially a guide to the entire literature on stress that has appeared since the publication of the first report in 1951. A summary of the concept of stress as it presents itself in 1952 is given. This is followed by a criticism of the principal objections against the stress concept. Thereafter follows a review of the literature on the (1) general physiology and pathology of stress, and (2) special physiology and pathology of stress.

W. J. HAMILTON

Histochemistry, Theoretical and Applied. By A. G. E. Pearse. (Pp. 530; 60*s.*) London: J. and A. Churchill Ltd. 1953.

The striking development of histochemistry in the past 10 or 15 years has resulted in an enormous increase in the literature of this subject. As a result, the necessity for a comprehensive histochemical work has become more and more acute. The pioneer work on histochemistry was that of Lison, published in 1936, and which at the time provided the only critical account of a variety of techniques of which many were of indifferent value and significance. However, the work, particularly on the histochemistry of enzymes, which has appeared since 1939 has rendered this valuable work sadly out of date, although a new edition is promised. Two American authors have produced books on histochemistry, and an English author one on cytochemistry in the last year or so, but these deal in detail with certain aspects, or certain branches of histochemistry, and make no pretensions to comprehensiveness. Dr Pearse has attempted to remedy this and has produced what might be described as a histochemist's Vade Mecum. However, the rapid pace of histochemical discovery not only makes it almost impossible for such a work to be up to date but also invalidates some of the comment about the techniques.

Dr Pearse has made a valiant attempt to cope with these and other difficulties which present themselves in compiling such a work and has produced a volume of 497 pages based on his own and other people's experiences of a variety of techniques. The book deals with the history of histochemistry, goes on to deal with fixation and then discusses the histochemistry of proteins, carbohydrates, lipids and lipoproteins, aldehydes and ketones, enzymes, pigments and inorganic constituents. A chapter is also devoted to physical methods of histochemistry, including autoradiography and there are full author and subject indices. Details of the various methods are given in the form of 89 pages of appendices. This is a most useful section.

In general, the comprehensiveness of the work is unquestioned, but when one comes to consider the statements contained therein some doubts concerning accuracy arise. Perhaps it is an unfortunate coincidence that in quoting the reviewer's work the author has not been very accurate. In one instance he claims that a reaction which was 'distributed more or less uniformly through the cell' was 'not intracellular'. In another instance he criticizes some work as requiring 'A stricter routine than that used', when in fact he has not seen the routine used, for it has never been published. In a third instance a number of his criticisms had been answered in another paper which he does not quote. Whether his comments in respect of other work have the same faults the reviewer cannot say, but some of his comments bear the stamp of immaturity of judgement.

Histochemistry is a subject which, because of its own peculiar difficulties and its speed of development, demands the most critical approach. Unless, however, such criticism is based upon (1) a careful reading, a reasoned appraisal and an accurate reporting of the work criticized, (2) a thorough knowledge of the related literature; the subject will be thrown into a state of confusion far worse than that which exists at present.

Most of the faults in Dr Pearse's book are due probably to the inherent difficulties of the subject, and their presence should not cloud the fact that he has produced a very valuable compendium of histochemical methods and a good deal of useful comment about them. The book is essential for the library of anyone interested in histochemistry. G. H. BOURNE

Some Problems in Neurophysiology. By F. Bremer. (Pp. 1–79; 21*s.*) University of London: The Athlone Press. 1953.

In 1950, Prof. Bremer, Professor of General Pathology in the University of Brussels, delivered three lectures at the University of London. These three lectures have been published with the help of Dr H. Davson of University College, who undertook the task of seeing the volume through the press. In this short monograph Prof. Bremer deals with synaptic transmission, inhibition and auto-rhythmicity in the spinal cord, the physiological basis of electroencephalography, and with oscillographic study of the auditory area of the brain. These topics are dealt with in an easy informative style that makes it possible for workers in other fields to follow the developments of this specialized work. It is perhaps a pity that three years have elapsed between the delivery of the lectures and publication, for the material, which appears substantially as it was first presented, does not therefore incorporate the more recent findings such as those of Eccles and his collaborators. There is a short supplementary note which to some extent rectifies these omissions. The fact that the subject-matter is discussed not only in its own right but to illustrate general principles of neurophysiology also makes the delay in publication of less importance. This book could be confidently recommended to the advanced student of anatomy or physiology, or to the research worker interested in this field. It clearly bears the hall-mark of being written by an authority. GEOFFREY HARRIS

Trends of Life. By F. WOOD JONES, F.R.S. (Pp. 191; crown 8vo; 10*s.* 6*d.*) London: Edward Arnold and Co. 1953.

In his introductory chapter the author states the aim and scope of this book: 'I shall be content to outline what is an individual view...as to what life seems to me to be and in what its ramifications, its progress, its changes and its adaptations appear to consist.' Unlike the statement in the preface of a much older book, dealing with the same problem, wherein its author writes, 'Simple the exposition cannot be, if one has any ambition for thoroughness', Prof. Wood Jones presents his views in 'ordinary plain English'. Indeed, the individuality, the clear exposition and the unorthodox nature of the views are all qualities which we have come to expect from this author, qualities which have, over the last fifty years, done so much to stimulate observation, thinking and discussion among English anatomists.

In the compass of just under 200 pages we have his views on vitalism, the purpose and directiveness of structural development, convergence, parallelism, correlation and domestication. With such a prominent scientist as Prof. Wood Jones upholding the theory of the transmissibility of acquired characters, it is impossible to agree with A. Russell Wallace's statement that 'the hypothesis of Lamarck has been repeatedly and easily refuted by all writers on the subject'. Developing this theory, Prof. Wood Jones finally turns to its implications concerning man himself. These are of profound significance for, as Herbert Spencer pointed out, on the acceptance or non-acceptance of Lamarckism must depend our views on life. Man is portrayed as domesticating himself and creating a situation in which 'viable pathological forms and generally abnormal types may survive and become perpetuated by artificial conditions'. Further, 'when the highly specialized, but limited, intellectual activities of the few are carried to extremes in their respective specializations, while the average intelligence of the masses is possibly on the decline, a state of society is produced that cannot be regarded as healthy'. Whether believing in the Darwinian or Lamarckian hypothesis or both, one is at times tempted to repeat the famous lines of Virgil:

'O gens
Infelix! Cui te exitio fortuna reservat?'

Prof. Wood Jones never writes a dull book nor an uninteresting article. His writings cannot fail to stimulate a reconsideration of the problem. This latest publication is no exception. The Lamarckian and Darwinian hypotheses are not mutually exclusive, and this small book will undoubtedly serve to focus some attention on the former view.

D. V. DAVIES

PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1952

The annual General Meeting of the Society for the Session 1952–3 was held in the Department of Anatomy, Charing Cross Hospital Medical School, Chandos Place, London, W.C. 2, on Friday, 28 November 1952. The President (Prof. W. E. Le Gros Clark) was in the Chair.

The following are the authors' abstracts of the papers presented.

Observations on the development and radiology of the Père David's deer foetus (*Elaphurus davidianus*). By T. W. Glenister. *Charing Cross Hospital Medical School, London*

Observations have been made on 10·5, 27·5, 33·5 cm. male foetuses and a 36·0 cm. female foetus. Dissection of the latter confirms, in general, Ray's work on the neonatus but some additional observations on the anatomy of the deer are made. The external form of the foetuses reveals that sensory vibrissae on the head appear some time in the 3rd or 4th month of gestation and that generalized hair growth starts at about the 6th month. It is noted that the testes reach the scrotum in the 5th or 6th month and observations are made on the dentition. Radiographs of the whole male foetuses and of the hemisected head of the female foetus show that ossification of the skeleton proceeds in a way similar to that of the sheep, and that the 10·5 cm. deer corresponds to an 8 cm. or 48-day-old sheep, that the 27·5 cm. foetus corresponds to an 18 cm. or 73-day-old sheep, whereas the 33·5 and 36 cm. foetuses correspond to sheep 20 and 21 cm. long and 80 and 92 days old. It appears that at corresponding stages of development, the deer is ahead in the ossification of its axial skeleton, but less advanced in the ossification of its limbs.

Observations in ova recovered from the oviduct and uterus of sheep. By R. Hadek. *University of Glasgow*

Eighty-one ova have been recovered from sixty sheep which showed signs of recent ovulation when examined after slaughter. Fifty of the ova were not segmented, thirty-one exhibited cleavage and six of the latter were considered to be abnormal.

In the ovarian third of the oviduct only non-segmented eggs were recovered and 50 % of these did not show any perivitelline space. It is postulated that in the freshly shed sheep ovum no perivitelline space is present. All other non-segmented ova exhibited a perivitelline space, the extent of which steadily increased as the ovum descended the oviduct. This feature was therefore most marked in non-segmented ova recovered from the uterus. In some of the latter the vitelline membrane had ruptured, and the cell contents were dispersed within the zone pellucida. These changes in the perivitelline space were accompanied by an alteration in the appearance of the yolk granules which exhibited an increase in size and a diminution in number. It is suggested that a degenerative process begins almost immediately after ovulation and is completed in the uterus.

No corona cells were found in any of the eggs.

Segmenting eggs were recovered from the oviduct in 2- to 10-cell stages. Some of the ova entered the uterus as early as the 8-cell stage.

In the 8- and 14-cell stage eggs, significant variations in the size of the blastomeres were noted. Blastomeres of three sizes could be distinguished, and it is suggested that some may have been early trophoblast cells and some dividing polar bodies.

Abnormal eggs were found in various stages of cleavage. In all these ova the outline of the blastomeres was distinct but only very few yolk granules were present. Variations in size of blastomeres were again noted in these abnormal ova.

The differentiation of the conducting system in the ventricles of the embryonic sheep. By A. R. Muir. *University of Edinburgh*

The ventricular conducting system has been examined in a series of fifty-one embryonic and adult sheep's hearts. The primordium of the common trunk appears first, at 9 mm., on the crest of the interventricular septum, as syncytial strands which have spherical, chromatic nuclei and are paler, broader and less striated than the normal myocardium. These modified strands are continuous with the normal myocardium of the interventricular septum; muscular continuity at other points on the atrioventricular ring is not definitely severed until 20 mm.

With increasing age the differentiation spreads into the myocardium of the ventricles. About 18 mm., the point of transition between modified tissue and the normal myocardium in the left branch of the bundle is on the left side of the interventricular septum, midway between the apex and the atrioventricular ring. In the right branch at about 20 mm., the transition occurs at the point where the right branch leaves the common trunk of the bundle; by 44 mm. the differentiation has spread to involve the musculature of the moderator band. A subendocardial plexus of differentiated tissue is present at 70 mm., and transitions can be seen between this modified plexus and the elements of the spongy myocardium. The intramyocardial plexus which appears as a further spread of the process of differentiation from the subendocardial plexus was not clearly observed until the foetus had reached 250 mm. in length.

The main and terminal branches of the atrioventricular bundle are developed from the ventricular myocardium by a peripheral extension of differentiation from the common trunk.

The preparation and properties of heart muscle sarcosomes. By K. W. Cleland. *University of Cambridge*

The sarcosomes (interstitial granules, mitochondria) of heart muscle lie in the sarcoplasm between the A-disks of the myofibrils. They contain about 25 % of the total protein of the muscle fibre and are responsible for most of the energy generation in the fibre.

Sarcosomes are readily separated from heart muscle and prepared in cytologically pure suspensions. Such suspensions are morphologically and biochemically unstable. After a short time certain enzymic functions are lost. After a longer time the granules undergo a series of morphological changes which are identical with those which occur immediately in granules suspended in hypotonic media. These changes indicate that the sarcosomes are composed of an elastic body gel surrounded by a membrane. Sarcosomes behave like semi-permeable systems.

At least some of the oxidative enzymes present in sarcosomes appear to be in the membrane.

Experiments on the longitudinal growth of muscles in rabbits. By G. N. C. Crawford. *University of Oxford*

The tibialis anterior of young rabbits was exposed at operation, small indian ink marks were made at intervals along the length of the surface of the muscle belly, and the distance between them measured. The rabbits were killed later, the distance between the marks again measured and hence the amount and site of longitudinal growth determined. Confirmatory experiments were performed by inserting very fine pieces of wire into the muscle belly and measuring the distance between these on radiographs taken at intervals during growth. These experiments show that interstitial longitudinal growth occurs throughout the length of the muscle belly.

A preliminary experiment was devised to test the hypothesis that the amount of growth in length of the muscle belly is determined by the distance it must contract in order to effect the full amplitude of movement of the limb. The tendon of tibialis anterior in young rabbits was transplanted in front of the divided crural ligament and the latter was sutured over the remaining extensor tendons. This increased the distance which tibialis anterior had to contract in order to dorsiflex the foot. Under these circumstances by the time the rabbit was fully grown, the experimental tibialis anterior muscle belly had become considerably longer than its normal counterpart of the opposite leg.

The fibre constitution of the human gastrocnemius and soleus muscles.

By E. W. Walls. *Middlesex Hospital Medical School, London*

The selective action of recently introduced drugs on red and white muscles in cats, together with electromyographic studies on human muscles reputed to be of different fibre make-up, have renewed interest in the relative proportions of the so-called red and white fibres present in the human calf muscles.

In twenty-four subjects the fibre content of the gastrocnemius and soleus muscles has been studied on frozen sections stained with many batches of Sudan III, Sudan IV and Sudan Black, and counts made of the fibre types present as judged by lipoid granule content. The relative sizes of the fibres has also been carefully checked, and it would appear that the granular fibres on average are slightly larger, although amongst both types there may be a wide range of fibre size. The decision with regard to typing individual fibres is frequently very difficult as granularity of different fibres varies from very heavy to nil. In the soleus the total granularity of fibres is usually noticeably heavier than in gastrocnemius. The naked eye appearances of the two muscles do not support current descriptions of gastrocnemius as a pale muscle and soleus a red one, nor does the myoglobin content of the muscles estimated spectroscopically indicate a consistent difference in this respect. The position of nuclei within the fibres does not help in classifying the muscles as red or white, as in both the great majority of nuclei are hypolemmal and in both the occurrence of nuclei within the fibres is only rarely seen. In longitudinal section appearances are met with which suggest a change in the degree of granularity of fibres along their length. This point was discussed.

The problem of compensatory hypertrophy and hyperplasia in the submandibular gland of the rabbit.

By W. D. Trotter. *University of Otago, Dunedin, N.Z.*

The changes which occur in the mitotic activity of the submandibular gland of the rabbit when the gland of the opposite side is removed have been studied quantitatively using the colchicine technique.

The mitotic rate in the submandibular gland of the rabbit is normally very low. In animals colchicined for 8 hr. an average of twenty-five mitoses in 50,000–150,000 cells occurred. In glands from the operated animals there was a 5- to 20-fold increase in mitotic activity, commencing on the 3rd day after operation, reaching a maximum between the 4th and 7th days and declining rapidly thereafter. In a minority of experiments no increase in mitotic activity was found. The age of the animal may be the factor responsible for this variability.

In a single experiment numerous mitoses were seen following dissection of the pedicle of the gland concerned.

An ancillary study was made of the changes which occurred in gland weights. In unoperated animals there is a considerable spread in the gland weight/body weight ratio, but the weights of both submandibular glands are very similar. In experimental animals there was a wider variation in the weight of the two glands, but neither was constantly greater.

These findings can neither confirm nor refute the hypothesis that a small degree of compensatory hypertrophy may occur in the rabbit submandibular gland, and a large number of more critical experiments would be necessary to solve this problem.

Ontogenetic changes in the colobid stomach. By W. C. OSMAN HILL. *Zoological Society of London*

The complex sacculated and capacious stomach of the monkeys of the family Colobidae (*Semnopithecus*, *Colobus*, *Nasalis*, etc.) has long been known (Wurmb, 1781; Otto, 1825; Owen, 1833 *et seq.*; Duvernay, 1835), but its ontogeny has not been worked out. Duckworth (1915) examined a single foetus (160 mm.) and made pertinent suggestions for further study.

It seemed logical to assume that early stages would reveal the process whereby, from a simple beginning, the complex form is evolved, possibly fairly late in foetal life, i.e. on the premise that the specialization has been acquired late in phylogeny. In view of opinions expressed by Keith & Wood Jones (1902) the study might throw further light on the curious features in human gastric ontogenesis.

Examination of a series of embryos and foetuses of *Semnopithecus priam* revealed that it is necessary to go back to a very early stage of development to find a really simple stomach, and even then there are indications of its future morphology. In the smallest possible embryo permitting macroscopic observations (9 mm.) a relatively simple gastric morphology is found, the organ consisting of an elongated pyriform sac oriented mainly in the long axis of the embryo. Nine later prenatal and a number of postnatal stages were considered in the translation to the complex form of the adult.

Observations were also made upon the correlated changes in hepatic topography and morphology.

Observations on the cochlea of certain lizards. By C. C. D. SHUTE and A. D'A. BELLAIRS. *University of Cambridge*

The auditory portion of the inner ear has been studied in prenatal and postnatal geckos—lizards which are noted for their ability to emit sounds. In these lizards the organ of Corti is relatively long, and the basilar and tectorial membranes are attached to a highly specialized supporting structure formed from condensed connective tissue which we have termed the limbus. It is comparable with the limbus and triangular ligament together in mammals. The tectorial membrane is suspended from a long lip projecting from the limbus and enclosing a concavity, the development of which is compared with that of the internal spiral sulcus of mammals. The perilymphatic duct system exhibits a posterior diverticulum lodged in a tunnel formed by a groove in the limbus which is spanned by a tapering extension of the basilar membrane. Since this portion of the basilar membrane is narrower than that situated more anteriorly, the anatomical arrangement in geckos may be such as to permit some degree of pitch discrimination. An identical type of limbic lip and perilymphatic diverticulum occurs in the superficially very dissimilar Pygopodidae, a family of snake-like lizards found in Australia, but in no other lizards examined. The phylogenetic implications of this observation are discussed.

Intrinsic visceral nerves. By G. A. G. MITCHELL. *University of Manchester*

By combining intravital (Weddell) and supravital (Schabadasch) methylene-blue techniques better staining of visceral nerves has been obtained than was secured with either method used by itself. At a subsequent meeting some of the findings may be discussed in more detail, but in this contribution slides will be shown of intrinsic nerves in the oesophagus, stomach, intestines, renal pelvis, ureter, bladder, prostate, seminal vesicles, trachea, bronchi, lungs, heart, aorta and arterioles.

For about 10-15 min. before being killed by a rapid overdose of intravenous Pentothal the animals breathed almost pure oxygen, and no fixatives were employed apart from the ammonium iodide and ammonium picrate recommended by Schabadasch. Before examination, the tissues were partly cleared in glycerine and mounted in a glycerine-picrate solution.

Appearances highly suggestive of a terminal reticulum were seen, especially in the subpleural, subperitoneal, aortic depressor, perivascular and subendocardial areas and they did not appear to be artefacts. In other areas, particularly in the bronchi, tracheobronchial junction, lungs and aorta complicated loops, whorls and nets were visible—probably receptors of various kinds.

The peripheral spread of nodal involvement in degenerating nerve.

By G. Causey and E. Palmer. *Royal College of Surgeons, England*

The first sign of degeneration in myelinated nerve fibres is the drawing apart of the ends of the myelin segments contiguous to each other at the node (Causey & Palmer, *Proc. Roy. Soc.* B, **139**, 1952). This distraction was observed in nodes very close to the lesion within a few hours. We have now investigated the mode of progression of this involvement of the nodes of Ranvier in the parts peripheral to the crush in the gastrocnemius and phrenic nerves of the rabbit.

Serial transverse sections (5μ) of these nerves were made. At different distances peripheral to the crush producing the degeneration, ten or more nodes were examined and the sections free from myelin staining were counted. At all times between 0 and 96 hr. the number of nodes at which the nodal gap between the myelin segments is greater than 5μ decreases along the nerve from the crush towards the periphery. On the other hand, the distance from the crush at which such large nodal gaps are found increases with the time allowed for degeneration, until at about 96 hr. increased nodal gaps can be shown in the nerve 8 cm. from the crush.

Observations on the development of microglia. By E. J. Field.

University of Bristol

The development of microglia has been followed in the mouse, rat and human brain and also in the postnatal rat and mouse. Contrary to the conclusion reached by Hortega (1920), fully ripened delicately branched microglial cells were found at an early stage (11 mm., rat; 13 mm., mouse; 55 mm., human—the earliest stage examined). These cells were large, isolated and not associated with the 'foamy' cells described by Hortega in his 'fountains'. Their derivation from meninges or blood vessels could not be established. Some branched cells with plump processes were found outside the nervous system, notably in the substance of the tongue. Small numbers of 'foamy' cells were found in the neonatal mouse brain, but they were more numerous and persistent in the rat at this stage. They occurred in the central white matter and below the corpus callosum. They were notably absent from the spinal cord and contained no fatty globules. They are thus not an obligatory precursor of microglia and seem to occur only in special situations between parts which are showing differential growth, and possibly result from some physical difference in the local ground substance of the brain.

Typingof the human sternum—an analysis of the ossification pattern in 520 sterna in the developmental stages. By G. T. Ashley. *University of Manchester*

In a previous communication (Ashley, *Proc. Anat. Soc.* 1950) based on the study of 100 adult East African sterna, I suggested a new method of classifying human sterna into three types according to the shape of the body of the sternum: type I, body of sternum narrow and with parallel sides; type II, body narrow above and wide below; Type III, body wide and with parallel sides.

From evidence derived from inspection and radiography of these sterna the view was put forward that the definitive shape of the body of the sternum is largely determined by the number and arrangement of the ossification centres from which this part of the sternum develops. In following up this problem the pattern of ossification in 520 sterna obtained at post-mortem from subjects in the developmental stages has been ascertained, i.e. from early foetal life to the age of 21 years.

The findings are analysed in relation to the method of typing adult sterna quoted, and the results of the investigation are shown to lend support to this method.

The arrangement of the smooth muscle in the extrapulmonary bronchi of the kitten. By B. Towers. *Department of Anatomy, University College, Cardiff*

Current text-books of Anatomy and Histology describe the structure of extrapulmonary bronchi as being identical with that of the trachea, and pay no attention to the interesting problem of how and where the changeover is effected from the well-known muscular arrangement in the trachea to that of the intrapulmonary bronchi.

The work of Guieysse (1898) on this question appears to have escaped notice, but since his technique was thought to be inadequate the matter has been re-investigated in the kitten by the study of serial sections, and the reconstruction from them of a model showing the relative dispositions of cartilage and muscle.

The model demonstrates that the muscle of the intrapulmonary bronchi is formed by modification of the trachealis muscle, the bundles of muscle-fibres gradually extending around the lumen of the bronchus in proportion as their 'cartilage of insertion' (i.e. of the tracheal type) diminishes. At the same time true bronchial cartilages appear in increasing amount outside the muscle bundles. This gradual mode of transition has been found, contrary to Guieysse's description, to be the same for both the bronchus to the lower lobe and that to the upper. The model also shows that in the region where the muscle first assumes the pattern characteristic of intrapulmonary bronchi there are internal projections of the bronchial cartilages to which bundles of muscle-fibres appear to be very closely anchored.

The epidermal melanocyte as a 'squamous' cell. By R. E. Billingham and P. B. Medawar. *University College, London*

Evidence was presented to show that the epidermal melanocyte (pigmentary dendritic cell) is a squamous or expendable cell, in the sense that some of its division products regularly pass outwards through the epidermis to be flaked off at the surface of the skin. It was shown *inter alia* that the 'clear cell' seen in orthodox vertical sections of the epidermis is the perikaryon of a melanocyte. The at present confusing terminology of the branched cells of the epidermis was discussed.

Some observations on the structure and development of Meissner's corpuscle. By N. Cauna. *King's College, Newcastle upon Tyne*

The structure, development and evolution of Meissner's corpuscle was studied by cytological, nerve-staining methods and plate reconstructions of skin from fifty-three individuals, ranging from late foetal life to 91 years.

Mature Meissner's corpuscles are cylindrical structures attached to the deep surface of the epidermis at angles of different degrees and directions. The papillary ridges have elevations continued from the axes of the corpuscles for selective transmission of the stimulus to the receptor. The horizontal striation of Meissner's corpuscle does not represent cell boundaries. Nerves entering the sides and base of the corpuscle form loops and circles dividing into extensive horizontal terminal networks.

The development of Meissner's corpuscle begins shortly before or after birth, and is first recognizable as a nerve termination on a lightly stained epidermal cell. Initial development is partly intra-epidermal (lightly stained portion), partly extra-epidermal (with dark nuclei). The difference between the two portions is gradually reduced and the corpuscle grows into the dermis. Nerve spirals appear during the initial stage of development, the terminal expansions after the first year. In individuals of middle age and older the corpuscles become coiled, lose their epidermal attachments and undergo reduction in size and number.

Meissner's corpuscles may represent modified epidermal receptors comparable with hairs. The corpuscle corresponds to the hair-sheath surrounded by nerves; the epidermal downgrowths around the corpuscle to the hair bulb and sebaceous glands; the epidermal ridge to the hair-shaft; the papillary pattern to the hair-tracts.

The presence of either Meissner's corpuscles or hairs singly or in groups, and the response of both with the same phasic reaction to a continued stimulus supports the above hypothesis.

Observations on the morphology and adrenaline-noradrenaline content of the abdominal chromaffin tissues. By R. E. COUPLAND. *University of Leeds*

The chromaffin tissue of the dogfish (*Acanthias vulgaris*), frog (*Rana temporaria*) and rabbit have been examined by histological and pharmacological methods. Fresh material was either fixed in formol-dichromate, sectioned, stained and examined microscopically, or was ground up with sand and N/10-HCl, centrifuged, and the supernatant fluid assayed for adrenaline and noradrenaline.

In the dogfish phaeochromocytes form segmental masses which are embedded in the mesonephros and associated with branches of the dorsal aorta; they are completely separate from the inter-renal body.

In the frog the chromaffin tissue is irregularly arranged on the ventral surface of the mesonephros and is associated with a small amount of adrenal cortical material, but does not form a medulla.

Chromaffin tissue is relatively abundant in the abdomen of the young rabbit and can be classified according to its relation to the adrenal gland as being either intra-adrenal (adrenal medulla) or extra-adrenal (para-aortic bodies). The two portions are continuous up to the age of 3 months.

Extracts were injected intravenously into chloralosed cats. Estimations of the adrenaline-noradrenaline content were made by comparing the effects on the arterial blood pressure and the contractions of the nictitating membrane with the effects of standards containing known amounts of these amines. The results obtained were as follows:

(1) Dogfish. Pressor amine: noradrenaline 100 %.

(2) Frog. Pressor amines: noradrenaline 55–70 %, adrenaline 30–45 %.

(3) Rabbit. (*a*) Adrenal medulla. Pressor amines: noradrenaline 8–13 %, adrenaline 87–92 %. (*b*) Para-aortic bodies. Pressor amine: noradrenaline 100 %.

Noradrenaline is a primary amine and adrenaline the *N*-methylated secondary amine: it is probable that noradrenaline is the normal precursor of adrenaline (Bülbring & Burn, 1949, *Brit. J. Pharmacol.* 4).

From the above results, it appears that the presence of adrenal cortical tissue in proximity to chromaffin tissue determines the appearance of adrenaline, whereas only the primary amine (noradrenaline) is found in chromaffin tissue not associated with cortical material.

Pulsation of the great veins recorded by high-speed cinematography. By D. A. McDONALD, E. P. W. HELPS and ALISON M. FERGUSSON. *St Bartholomew's Hospital, London*

The exposed rabbit heart has been filmed with an Eastman-Kodak high-speed cine-camera at 1500 frames/sec. Illumination was from a single concentrated source lamp, focused by a condenser and shining through heat-filter glass. On projection movement is slowed *c.* 100 times so that the sequence of all phases of the cardiac cycle may be seen. Pulsations are observed in the large venous trunks; the most marked are those of the left anterior vena cava. The timing of the pulsation is such that emptying of the vein occurs late in atrial systole and is clearly due to active contraction. Dilatation of the vein occurs during diastole and it fills at the same time as the atrium. Reflux into the vein from the heart plays a major role in distending the vein and in one sequence this reflux appears to begin during ventricular systole. Movements of the vein can be seen with the pericardium intact or after opening it, and the pulsations are almost as large after ligation of the vein at the level of the arch of the aorta. The region pulsating usually extends somewhat beyond the pericardial attachment. Histological preparations show that there is cardiac muscle surrounding the region of the vein that shows active contraction.

The cinematographic expenses were defrayed by a grant from the Medical Research Council to D. A. McDonald.

FEBRUARY 1953

An ordinary meeting of the Society for the Session 1952–3 was held on Friday, 27 February 1953, in the Department of Anatomy, St Mary's Hospital Medical School, Paddington, W. 2. The President (Prof. W. E. Le Gros Clark) was in the Chair.

The following are the authors' abstracts of the papers read.

Some observations on the amygdaloid complex of the porpoise.

By A. S. Breathnach. *St Mary's Hospital Medical School, London*

Le Gros Clark & Meyer (1947) and Meyer & Allison (1949), working on the rabbit and monkey respectively, found that olfactory fibres terminated in the cortico-medial, but not in the baso-lateral group of amygdaloid nuclei. These findings, taken together with the demonstration by Crosby & Humphrey (1941) that in microsmatic man the cortico-medial group is overshadowed by the baso-lateral group, indicate a functional distinction between the two.

In the anosmatic porpoise, the baso-lateral group is highly developed and in its degree of differentiation closely approaches the condition found in man and other primates. By comparison, with the exception of the cortical nucleus, the cortico-medial group as a whole is poorly developed, especially the medial nucleus, while the nucleus of the lateral olfactory tract is barely, if at all recognizable. The central nucleus is moderately well developed.

These findings suggest that the amygdaloid complex of the porpoise has undergone a development which closely parallels that undergone by the corresponding formation in man, and that the governing factor common to both has been the reduction in the olfactory sense. The fact that the cortical nucleus appears to be better developed in the porpoise than in man would appear to bear this out, as it has been shown (Rose, 1927) that this nucleus is better developed in microsmatic man, than in the general run of macrosmatic mammals.

Some neurological features of a microsmatic lizard.

J. A. Armstrong, H. J. Gamble and F. Goldby. *St Mary's Hospital Medical School, London*

The lizard *Anolis carolinensis* has been described as microsmatic, and the statement has appeared that the small olfactory bulbs differ from those of other reptiles in that they are present only as minute conical projections on the under surface of the frontal poles of the hemispheres, and that accessory olfactory bulbs are absent (Crosby & Humphrey, 1939). Pratt (1948) has made the further statement that epithelium of the vomero-nasal organ is non-sensory in character.

Through the co-operation of Dr G. Underwood of the University College of the West Indies we have had the opportunity of examining the condition of these structures in three species of *Anolis: A. lineatopus, A. garmani* and *A. grahami.*

The microsmatic character of these animals has been confirmed, but typical though small olfactory and accessory olfactory bulbs are found to be present attached to the hemispheres by long peduncles as in other Lacertilia. Excision of these bulbs causes characteristic degeneration in the neuro-sensory epithelium of both the nasal sac and the vomero-nasal organ.

In the hemispheres it is found that parts which normally receive olfactory connexions are markedly reduced, and in particular that the anterior part of the pyriform cortex is represented only by a vestige. Much of the dorsolateral pallium is represented in these animals by a membrane containing neither nerve cells nor fibres.

Nuclear population changes in the degenerating posterior columns of the rabbit's spinal cord. By J. Joseph. *Guy's Hospital Medical School, London*

Nuclear population changes in degenerating peripheral nerves vary with the size of the fibres, the larger the fibres the greater is the increase. Quantitative studies of the nuclear population in degenerating tracts of the spinal cord have been few and contradictory and failure of regeneration has been linked with absence of multiplication.

One posterior column of the rabbit's spinal cord was sectioned in the lower cervical region, and the nuclear changes following degeneration were studied after intervals of 10, 20, 50 and 100 days. After 10 days the increase was found to be about 1·8 times, after 20 days about 3·0 times, after 50 days about 3·4 times, and after 100 days about 4·5 times. This increase is much smaller than in peripheral nerves of similar fibre size. In addition, these nerves show their maximum increase after 25 days whereas the spinal cord tract nuclei continue to increase for a much longer period.

This increase is due to phagocytosis in the early stages and the formation of a glial scar at a later stage. It is suggested that the multiplication of the cells affects astrocytes and oligodendrocytes equally at 10 days, that at 20 days the oligodendrocytes increase more than the astrocytes but that subsequently the astrocytes multiply much more. The cells were distinguished by their nuclei.

There is no reticulin in the tubes of the spinal cord nerve fibres, so that, unlike peripheral nerves, degeneration does not leave fairly rigid tubes. It is probably this which accounts for some of the differences between spinal cord tracts and peripheral nerves during degeneration and also the failure of functional regeneration in the spinal cord.

Experimental studies on the descending tectal pathways of the cat. By G. W. Pearce (*University College, Dundee*) and P. Glees (*University of Oxford*)

An experimental study of the tectum of the midbrain of the cat has been carried out to determine the course and distribution of the tecto-spinal and other descending tectal tracts and their relationship to the cells of the reticular formation.

It has been determined that the tecto-spinal tract is in two divisions, crossed and uncrossed, and that it originates from the superior corpus quadrigeminum and extends distally to the eighth cervical and first thoracic segments. Fibre counts, and an examination of Marchi and silver preparations, have shown that the crossed tecto-spinal tract loses more than half of its fibres between the inferior corpus quadrigeminum and the medulla, and that most of the fibres lost pass to the reticular formation, only a few to the nuclei of the cranial nerves supplying the muscles of the eyeball. The relationship between the tecto-spinal fibres and the medial longitudinal fasciculus is described. This paper is based mainly on the study of Marchi preparations.

Hair pigment patterns. By R. L. Holmes. *University of Leeds*

The disposition of melanin pigment in hair varies in the cortex and medulla. Those hairs which are predominantly cortical in structure (e.g. human) show pigment granules lying in elongated groups along the axis of the hair. Those in which the medulla is well developed (e.g. rat, mouse) show banding, due to an aggregation of pigment granules in bands in the medulla, each band being separated from the next by a clear area; cortical pigment in such hairs conforms to the usual pattern.

The development of this arrangement is a direct result of the specific localization of pigment within epidermal cells (nuclear capping), and is a manifestation of a general characteristic of such cells. Cortico-medullary differences in pigment pattern are due to the separate lines of development undergone by matrix cells forming the hair cortex and medulla respectively.

The organization of dendrites in the neurones of the visual and motor cortices of the cat brain. By D. A. Sholl. *University College, London*

The dendrites of a number of cells from the visual and motor cortices of the cat have been studied by measuring the lengths, diameters and directions of all their branches in thick sections stained by the Golgi-Cox technique. The numbers of branches at increasing distances from the perikarya were found and the numbers of dendrites intersecting a unit area at the same distances calculated. The branching pattern differs in the motor and visual areas. The density of intersections falls off exponentially with increasing distance, but less rapidly in the motor cortex than in the visual. In the motor cortex the branches are fewer and longer.

It is highly probable, as suggested by Cajal, that differences between dendritic organization are important factors in the activity of the nervous system. This method provides quantitative means for assessing differences of this organization not only between different parts of the cortex of the same animal, but between different species of adult animals, between animals of the same species at different ages and between normal and pathological conditions.

The main object of this work, however, is to provide preliminary tests for hypotheses concerning the modes of activity of the visual cortex. Some of these have been suggested by the theory of an analogue machine (Sholl & Uttley, *Nature, Lond.*, 1953) capable of distinguishing between a presented pattern and a stored ensemble of patterns. The theory of this machine predicts, *inter alia*, random connexions between the input and the units of the machine governed by an exponential probability law. These studies show that the prediction appears to be fulfilled and enable the parameters of the law to be calculated.

Observations on the chromaffin reaction. By R. E. Coupland. *University of Leeds*

For approximately one hundred years the chromaffin reaction has been used in studies on the adrenal medulla. A brown coloration results when tissues which contain adrenaline or noradrenaline are fixed in formal-dichromate. Initially this brown coloration was thought to be due to the fixation of chromium in the medullary cells.

Lison and associates later advanced the view that the reaction was an oxidative effect and independent of chromium.

In the present investigation analytical chemical methods and radioactive chromium (^{51}Cr) have been used. The results indicate that chromium is fixed by the medullary cells, and the view is advanced that the reaction consists of an initial oxidation with the subsequent fixation of chromium.

The effects of various methods of fixation on the gram reaction in the basiphil granules of the pituitary. By R. R. Wilson and C. L. Foster. *St Mary's Hospital Medical School, London*

We previously reported (*Proc. Anat. Soc.*, Feb 1951) that Gram-positive staining of basiphil granules in the pituitary is probably not due to ribonucleic acid. Fixation may affect such findings, and this communication reports the results of a study of the Gram reaction in the basiphil granules after various fixatives, as well as in unfixed and freeze-dried material. The gram reaction was positive after fixation in formaldehyde-saline, Helly's fluid, Zenker's fluid, and Zenker Base. Unfixed and freeze-dried material was

Gram-negative, but sometimes became Gram-positive if the sections were left in formaldehyde-saline for about 12 hr. before staining. All other fixatives tried gave negative results. Staphylococci were Gram-positive by all methods of fixation.

Histochemistry and cytology of salivary gland duct cells. By C. Ruth Hill and G. H. Bourne. *London Hospital Medical College, London*

Various histochemical and cytological techniques have been applied to the submandibular and parotid glands of rabbit, rat, cat, guinea-pig and mouse and the sublingual glands of rabbit, rat, and cat. Gomori's naphthol AS-acetate and α-naphthol methods and Seligman's β-naphthol method were used to investigate the presence of simple esterases. In many cases striking results were obtained which showed high esterase activity in the cytoplasm of the duct cells, whilst the secretory acini gave only a faint positive reaction or were quite negative. The reaction was in some cases diffuse, whilst in others it took the form of discrete granules, often confined to either the distal or basal cytoplasm. In some glands the interlobular gave a much stronger reaction than the intralobular duct cells.

The nuclei of the duct cells gave a fairly strong positive reaction for acid phosphatase in most cases, whilst the cytoplasm gave a weaker diffuse reaction. No alkaline phosphatase activity was discovered, though in two cases the duct contents gave a positive reaction. Very little lipase activity was demonstrated. The PAS test for polysaccharides gave a positive result around the edges and in the Golgi region of some duct cells. In the few glands examined, the duct cells contained large amounts of both mitochondrial and Golgi material.

Our results sustantiate the view expressed as far back as 1866 by Pflüger, that the ducts are not merely passive conducting tubes but that they are metabolically very active and may indeed possess secretory or absorptive functions.

Ribonucleoprotein and the structural changes of mitosis. By J. Boss. *Middlesex Hospital Medical School, London* (introduced by E. W. Walls)

Living newt fibroblasts in tissue culture were observed and photographed by phase-contrast microscopy. After fixation, cultures were stained either with methyl green and pyronine or with May-Grünwald and Giesma's stains. The distribution of nucleoproteins was compared with structural changes in the dividing cell and the following points noted.

Ribonucleoprotein is a constituent of some of the granules found among the chromonemata in the interphase nucleus, and, during prophase, is deposited or formed on the chromosomes. The nucleolus, which is rich in this protein, subsequently breaks down, its dissolution being accompanied by the rounding up of the cytoplasm. In metaphase each chromosome consists of two chromatids embedded in a matrix of ribonucleoprotein. During anaphase, elongation of the spindle and cell cleavage begin together, and, at the same time, the separating groups of chromosomes shed ribonucleoprotein which is left in the equatorial region of the cell. In late anaphase, and in early telophase the cells show transient surface irregularities. This 'bubbling', which is a normal feature of mitosis in whole tissues, is probably caused by ribonucleoprotein shed from the chromosomes.

Thus, the rounding up of the cell, the onset of cleavage, the elongation of the spindle and the transient surface irregularities of the mitotic cell all coincide with a change in the distribution of ribonucleoprotein; almost certainly this is the agent bringing about at least the last of these four changes.

Glycogen in cellular differentiation. By R. J. O'Connor. *Westminster School of Medicine, London*

Determination of the number of dividing cells in the liver of the chicken embryo reveals a considerable decrease as glycogen appears. Previously recorded results show that, at the same time, there is a loss of the ability of the liver cells to catabolize glucose by respiratory mechanisms and that differentiation begins, in the sense that adult functions can be demonstrated. It is claimed that the results of other investigators show that there is a similar association of events in the formation of cutaneous epithelium. On the basis of this similarity the significance of glycogen in cellular differentiation was discussed.

The early development of the acoustico-facialis complex of ganglia in a lamprey. By A. Fisk. *St Mary's Hospital Medical School, London*

The acoustico-facialis complex of ganglia in a lamprey is shown to be formed partly from the ear placode itself, and partly from the neural crest. The neuromast ganglia of the head, which constitute a part of the complex and which lie outside the ear capsule, originate from the cranial part of the ear placode. The somatic sensory and visceral sensory parts of the intra-capsular ganglionic mass form from the neural crest. The more caudal part of the intra-capsular mass, which innervates the ear and is the acoustic ganglion, is derived from the ventral part of the ear placode. The regions of the ear vesicle which give origin to ganglion cells remain thicker than the rest of the wall of the vesicle and form the macula acoustica communis. The ear placode does not contribute to the glossopharyngeal ganglion.

Studies on the circulation in autografts and homografts of rabbit skin.
By R. J. Scothorne and I. McGregor. *University of Glasgow*

The revascularization of full-thickness, fitted autografts and homografts of ear skin in rabbits has been studied by repeated intravenous injections of 4% bromophenol blue. Skin with intact circulation is immediately coloured blue following injection; skin grafts prior to vascularization are not immediately coloured. Using this method it has been established that both autografts and homografts are fully revascularized by about 4 days following the placing of the graft. This is contrary to the findings of Ham (*J. Bone Jt. Surg.*, **34**, 1952) and of Conway *et al.* (*Plast. Reconstr. Surg.* **9**, 1952) who maintain that homografts of skin never become vascularized at all. At some variable time after the re-establishment of the circulation, homografts are destroyed. Failure of the circulation—indicated by failure of bromophenol blue to enter the graft—is noted at the same time as histological signs of epidermal destruction. Since skin grafts can and do survive for periods of up to four days prior to revascularization, it is unlikely that the destruction of homograft epithelium is due to this simultaneous vascular breakdown.

The growth of the embryonic neurite in tissue culture.
By A. Hughes. *University of Cambridge*

In 1907, Ross Harrison observed the growth of nerve fibres from explanted Amphibian neuroblasts, and described the pseudopodial activity of the tip of the growing fibre. By analogy with amoeboid movement, it has been suggested that these pseudopodia are concerned with elongation of the fibre, though study by phase-contrast ciné-micrography of the living neurite in culture casts doubt on this interpretation. From time to time, vacuoles of the external fluid enter at the tip, as Warren Lewis has already observed; moreover, he has pointed out the resemblance which this process bears to the 'pinocytosis' of a macrophage, by the activity of its undulating membrane. The vacuoles in the neurite can be seen to pass along the fibre towards the cell body.

If the neurite is severed where it emerges from the explant, the distal portion, no longer connected to its cell body, continues to elongate for a period up to three hours. It seems, therefore, that growth can still continue for this period in the absence of any flow of fresh neuroplasm down the neurite from the cell body.

The anatomy of hare-lip in man. By T. Summerfield King. *University of Sheffield*

Two specimens of bilateral complete hare-lip with cleft palate in full-term foetuses, one sectioned serially and the other dissected, were compared with normal sectioned and dissected material.

Graphic reconstructions showed the presence of premaxillae with sockets for the incisor teeth and subvomerine processes, but no palatal or facial processes.

The arterial supply of the median tubercle was derived from the left facial artery by an arching trunk which crossed the nasal bone to reach the mid-line and then traversed the columna and philtrum. The nerve supply was from the infraorbital nerves by large trunks which passed on to the alae of the nose and then through the columna to reach the tubercle. The incisor teeth had a copious blood supply, but no nerve supply. Anterior superior dental nerves traversed the maxilla, but emerged from its facial surface and joined the infraorbital trunks. Study of the specimens leads to the conclusion that the distribution of sensory nerves cannot be used safely for determination of embryonic sources of adult tissues.

A visual display method of recording progress in the dissecting room. By D. C. Sinclair and H. D. Darcus. *University of Oxford*

A system of supervising and controlling routine *viva voce* examinations during the term by means of an appointments list and a visual display chart of the kind used in industry has been introduced at Oxford. The method of operation and advantages of the system were discussed.

The development of the mammalian dural venous sinuses. By H. Butler. *St Bartholomew's Hospital Medical College, London*

In many mammals the dural venous sinuses open into the external jugular vein via the postglenoid foramen (the foramen jugulare spurium of Luschka) and into the internal jugular vein via the jugular foramen. The literature contains no account of the development of the postglenoid foramen and its contained vein is said to be the persistent vena capitis lateralis (Streeter, 1915). In the rat the main route of dural venous outflow is via the postglenoid foramen; the development of these sinuses was therefore investigated in rat embryos ranging from 11 days to full term (1·6–20·0 mm. c.r. length).

It is shown that the vena capitis lateralis commences opposite the geniculate ganglion and runs caudalwards embedded in the chondrocranium and lying in a plane deep to the dermocranium until it reaches the position of the developing styloid process. It then runs upon the outer surface of the mastoid process of the otic capsule and turns medialwards, with Arnold's nerve, to join the internal jugular vein. Opposite the squamotympanic fissure two veins join the commencement of the vena capitis lateralis:

(1) Dorso-medially the petro-squamous sinus which runs upwards in an oblique canal in the orbito-parietal commissure (the canal of Verga) to join the transverse sinus at the point of entry of the superior petrosal sinus.

(2) Laterally, an emissary vein which runs lateralwards anterior to the cranial end of Meckel's cartilage and posterior to the temporo-mandibular joint to join a tributary of the external jugular vein. This is the postglenoid vein.

These two veins, united by the vena capitis lateralis, rapidly increase in size and become the main route of outflow for the dural venous sinuses. The major part of the vena capitis lateralis which extends caudalwards in the facial groove of the otic capsule, disappears completely.

The narial muscles of Crocodilia. By A. d'A. Bellairs and C. C. D. Shute. *University of Cambridge*

Among other special modifications for amphibious life crocodiles possess a system of unstriped muscles, probably unique among amniotes, by means of which the nostrils can be opened and closed. There are two distinct muscles related to each nostril, a longitudinally disposed dilator, attached to the posterior margin of the narial opening in front and to the premaxilla and nasal bones behind, and a circular constrictor muscle which completely surrounds the dilator in sphincter-like fashion and is attached to the narial margins. The constrictor probably acts mainly by compressing and squeezing forwards the dilator muscle, so that the posterior margin of the nostril becomes apposed to the anterior margin. The innervation of these muscles was studied in de Castro preparations. So far as can be ascertained, their innervation is derived entirely from the sympathetic system; post-ganglionic fibres originating in the superior cervical ganglion are distributed to the muscles by way of the ophthalmic nerves.

MAY 1953

An ordinary meeting of the Society for the Session 1952–3 was held on Friday, 1 May 1953, at St Thomas's Hospital Medical School, London, S.E.1. The President (Professor W. E. Le Gros Clark) was in the Chair.

The following are the authors' abstracts of the papers read.

The anatomy of the abdominal viscera in Propithecus. By D. V. Davies. *St Thomas's Hospital Medical School, London*

A brief account of the arrangement of the abdominal organs in Propithecus is given. The stomach is placed almost entirely under the left costal margin with the pyloric half facing ventrally. The duodenum is long but presents little that is unusual. The remainder of the small intestine lies on the left side of the abdominal cavity, the major part of which, behind the liver and stomach, is occupied by the colonic labyrinth. There is still disagreement in the literature concerning the precise disposition of the coils of the colon. In the adult these are arranged in two spirals, placed ventrally and dorsally in contact with one another and wound around a central loop belonging to the dorsal spiral. The caecum is not coiled spirally along its long axis in the adult. The arrangement of the blood vessels to the colonic labyrinth gives some indication of its mode of development.

Distribution and fate of radio-active sulphate in the connective tissue of the rat. By D. V. Davies and L. Young. *St Thomas's Hospital Medical School, London*

^{35}S labelled sodium sulphate was injected intraperitoneally into rats. The animals were killed at varying time-intervals after the injection and the distribution of ^{35}S in the connective tissues was studied by radio-autography supplemented by estimations of radio-activity by the usual counting procedures. In cartilage there was a marked accumulation of ^{35}S, most noticeable in the hypertrophic zone of epiphyseal cartilage. Washing of this tissue in water and in an alkaline buffer solution (pH10) failed to remove much of the radio-active material. The significance of this was discussed.

The concentration of radio-active alloxan in the tissues of the rat. By R. G. Janes. *St Thomas's Hospital Medical School, London.* (Introduced by D. V. Davies)

Alloxan monohydrate labelled in either the 2- or 5-position with ^{14}C was given subcutaneously to adult rats. The concentration of this material in tissues was determined at intervals from 5 min. to 6 hr. and the excretion rate up to 22 hr. The radioactivity of the kidneys and plasma was the highest and reached its peak in about 15 min. The other tissues studied, including the pancreas, had very low ^{14}C concentrations. Approximately 95 % of the labelled alloxan was excreted in 22 hr. Only minute amounts of radioactivity appeared in expired air or as urea. Evidently the toxic effect of alloxan on pancreatic islets is dependent upon the specific sensitivity of beta cells and not upon an excessive concentration of this material in the organ.

The histochemistry of xanthide oxidase. By G. H. Bourne. *London Hospital Medical College*

Xanthine oxidase is responsible for the oxidation of the xanthine and hypoxanthine derived from the guanine and adenine of nucleic acid to uric acid.

Liver, kidney, spleen and intestine have been shown biochemically to be the organs which possess the highest xanthine oxidase activity.

This enzyme has not previously been localized histochemically, but the fact that it is a dehydrogenase suggested that it might be demonstrated by the use of neotetrozolium in the way that succinic dehydrogenase has been localized.

Frozen sections and thin slices of fresh unfixed tissues were incubated in an atmosphere of nitrogen for 1 hr. in a mixture containing pyrophosphate buffer, xanthine and neotetrazolium. The neotetrazolium was reduced to an insoluble diformazan pigment by the hydrogen liberated by the xanthine oxidase from the xanthine, and was precipitated at the site of enzyme activity.

In all tissues the nuclei were found to be completely negative and in the gut the brush borders were negative. Cells in the red pulp of the spleen (which appeared to be reticular cells) gave a positive reaction. In the liver the peripheral cells of the lobules were more active than the central cells. In the kidney the greatest reaction was given by the cells of the proximal convoluted tubules and the descending limbs of Henle's loops. A less intense reaction was given by the other tubules. A slight reaction was given by the glomeruli. In the intestine all the epithelial cells of the villi were strongly positive, those of the crypts less so. The positive reaction consisted in each case of a diffuse red background (regions of low activity) and a bright blue granular reaction (regions of high activity). Both these reactions were distributed uniformly through the cell.

The effect of adrenalectomy and injection of steroid hormones on the succinic dehydrogenase reaction of tissues. By H. A. Malaty and G. H. Bourne. *London Hospital Medical College*

Succinic dehydrogenase can be demonstrated in tissues by incubating fresh frozen sections in a substrate containing succinate, phosphate buffer and neotetrazolium. The positive reaction is dark blue and sites of lower activity show as pink or red. This reaction is widespread in tissues and is found to be particularly active in kidney, heart, muscle, liver and brain. The injection of cortisone into intact animals causes an increased intensity in this reaction in all four tissues but adrenalectomy causes a substantial decrease. Injection of cortisone into adrenalectomized animals returned the reaction to near normal in liver, heart and brain; injection of desoxycorticosterone returned the reaction almost to normal in the kidney. Injection of both hormones returned the reaction in all tissues almost to normal. Injections of testosterone into adrenalectomized animals had no effect, and oestrone either had no effect or depressed the reaction. It was noteworthy that in normal and adrenalectomized animals the injection of cortisone caused a disappearance of the succinic dehydrogenase reaction from the intercalated discs of heart muscle.

Further observations upon the axis of rotation at the human ankle joint. By C. H. Barnett. *St Thomas's Hospital Medical School, London*

The axis of rotation at the human ankle has been determined experimentally by modification of Manter's method (*Anat. Rec.*, **80**, 1941). Although its inclination to the horizontal changes as the foot moves up and down, the axis remains throughout in an oblique vertical plane that passes approximately through the medial and lateral malleoli. It bears no constant relationship to the long axis of the trochlear surface of the talus, nor to the long axis of the foot. It appears, however, that the obliquity of the axis at the ankle is related to the obliquity of the metatarsal heads, as estimated from standard radiographs of a number of normal subjects. The significance of this relationship is discussed.

Electromyographic study of the external anal sphincter in man. By E. W. Walls and W. F. Floyd. *Middlesex Hospital Medical School, London*

By the use of surface electrodes the action of the external anal sphincter has been studied in seven normal male subjects. Despite the sensitivity of the anal skin the contact of the electrodes is soon quite unnoticed and it seems probable that reflex activity due to their presence is unimportant. The following observations were made:

During the waking state the muscle is in a state of tonic contraction to a degree which varies from person to person and which varies greatly with different forms of activity. To a considerable extent the sphincter is under the control of the will and by voluntary effort can be made to contract vigorously. Its associated activity with that of the gluteal muscles was discussed. With increase of intra-abdominal pressure, from whatever cause, there is increase of sphincter tone, but when such increased pressure accompanies an attempt at defaecation relaxation of the sphincter can be demonstrated in some subjects. The response of the muscle to digital examination is brisk and vigorous. The functional properties of the sphincter was discussed in the light of observations made on its histological structure.

The articular surfaces of the sacro-iliac joint.
By H. Weisl. *Manchester University*

Using cartographic methods the outlines and contours of sixty-two sacro-iliac joints were examined. Considerable variation was found in the outline of the sacral articular surface. The shape of the ventral border is related to the sacral curvature and the slope of the ala; whilst the concavity of the dorsal border is more marked in females and changes with age. The sacral surface is proportionately longer and narrower than the corresponding iliac surface.

On the sacral articular surface there are usually two main elevations: a cranial, on some part of the lateral aspect of the first sacral vertebra, and a caudal, which is smaller. These two elevations are separated by a saddle-shaped depression in young subjects. The sacral articular surface is also hollowed out dorsal to both the cranial and caudal elevations. The iliac articular surface is reciprocally shaped, but the two surfaces are not entirely congruent.

Ageing of the subject is associated with roughening and fissuring of the articular cartilage and with an increase in the difference in height between the highest and lowest parts of the articular surface. Near the dorsal border an elevation, sometimes of considerable size, is formed, and others appear on the ventral border. These changes diminish the mobility of the joint and culminate in its ankylosis.

Voluntary dislocation of the shoulder.
By H. Hughes. *University College, Cardiff*

A young adult male discovered, by accident, that he could dislocate his right shoulder at will. There is no history of any injury. Dislocation is effected by moderate abduction and medial rotation of the humerus followed by contraction of the long head of triceps and the infra-spinatus muscle. The head of the humerus is withdrawn from the glenoid fossa and displaced posteriorly. The bones are replaced by contraction of the central part of the deltoid muscle. No abnormality can be detected in radiographs, and it would appear that the dislocation is dependent upon some defect in the capsule or the articular cartilage. That this is congenital in origin is suggested by a family history of some similar defect in the right shoulder. The reason for the withdrawal of the head of the humerus from contact with the scapula is not apparent.

The paper is illustrated by a short cinematograph film.

A study of the choroidal circulation of the eye in man. By K. C. Wybar. *Institute of Ophthalmology, London.* (Introduced by W. J. Hamilton)

The pattern of the choroidal circulation is demonstrated by intravascular injection of Neoprene latex, the elastic nature of which permits dissection of individual vessels from the main vascular mass.

The choroid is supplied by two arterial systems: First, the short posterior ciliary arteries which divide into many branches as they pierce the sclera around the optic nerve and terminate in arterioles which open into the chorio-capillaris. Each branch confines its arteriolar-capillary network to a localized zone of the choroid between the optic disc and the equator of the eye. Secondly, the recurrent choroidal arteries which arise from the long posterior ciliary arteries, from the anterior ciliary arteries and from the major arterial circle of the iris, and which supply the anterior part of the choroid meeting the short posterior ciliary arteries near the equator. The junction of the two systems may be marked by an intervening capillary network, but it is not uncommon for a direct branch to effect the union and in certain cases the vessels may inosculate.

The capillaries in the anterior part of the chorio-capillaris are larger than those in the posterior part, and the intercapillary meshwork is more open anteriorly. There is no evidence of any arterio-venous anastomoses in the choroid.

A note on the nature of the nerve cells of the ciliary ganglion. By R. Warwick. *University of Manchester*

In 1932 Kiss claimed to have distinguished in autonomic ganglia, including the ciliary, two types of nerve cell, one large and pale (which he considered sensory), the other small and darkly stained (which he thought sympathetic). Both types were depicted in the ciliary ganglia of Man and the macaque, the small cell predominating.

Ranson & Fisher (1933) showed that the prolonged osmic acid technique used by Kiss could produce gross artefacts, and they did not substantiate his conclusions. In material stained by cresyl fast violet their histological findings were corroborated in the present research. In addition, the results of experimental interruption of the short ciliary nerves, of removal and evacuation of the eyeball, and of irridectomy, which were carried out on five cats and ten monkeys (*Macaca mulatta*), demonstrated clearly that Kiss's view of the ciliary ganglion as a mixed population of sensory and motor nerve cells was incorrect.

Observations on the nervous component of the atrioventricular bundle in the cat, and on the innervation of the myocardium. By E. J. Field. *University of Bristol*

Large numbers of nerve fibres, both individually and collected in trunks, enter the proximal part of the bundle and course within it. The great majority of these nerves forsake the bundle to pass into the upper part of the intraventricular septum, only a few individual fibres passing into the proximal parts of the bundle branches, especially the right. The bundle branches themselves spread out as thin sheets beneath the septal endocardium; continuity with ventricular musculature could not be established. In one heart a group of ganglion cells was found in the hollow of the bundle bifurcation where it straddles the interventricular septum. No nerve endings could be made out with certainty in the bundle or its branches. Numerous nerve fibres, both in bundles (particularly around blood vessels) and singly, could be found in the septal myocardium. Some very fine nerve-fibres appeared to pass within heart muscle cells often in apposition with a nucleus. Appearances suggesting a juxtanuclear termination were sometimes encountered, but this could never be established with certainty because of the high magnification at which observations had to be made. There is, however, certainly present a network of nerve fibres which, if they do not actually run intracellularly at least in part, comes into very close relation with heart muscle cells. It is suggested that a nervous impulse set off in this network might activate the myocardial cells, the phenomenon being similar to that recognized by physiologists in nerve-muscle preparations and hinted at by Young (1933) as obtaining in the sympathetic ganglia of selachians.

Some observations on the nature and functions of the papillary ridges of the skin. By N. Cauna. *King's College, Newcastle upon Tyne*

In a recent communication (Cauna, November 1952) it was suggested that the primary function of the papillary ridges of the skin was sensory, and that they were concerned with the selective transmission of touch stimuli to the receptors (Meissner's corpuscles). An account was given of the structure of the papillary ridges in man and their relationship to the underlying Meissner's corpuscles. Their development and mode of function was discussed.

Observations on the progressive growth of the dental lamina.

By K. M. Backhouse. *King's College, Newcastle upon Tyne*

The development of the dental lamina and the enamel epithelium has been studied in human and comparative material. Reconstructions to show the dental epithelial structures have been made in 28 and 44 mm. c.r. length human embryos. The present study has been restricted to the canine and incisor regions in view of the relatively simple pattern of these teeth. The growth of the dental lamina is characterized by waves of activity on the labial aspect of the lamina which produce ridges in the areas of the future teeth parallel to the lip furrow band. The first ridge formed usually develops to a variable extent and then disappears. The second ridge develops to form the labial half of the enamel organ of the future milk tooth. The lingual half is still represented by the undifferentiated tip of the dental lamina. This will subsequently split to form the lingual half of the enamel organ of the milk tooth and the permanent tooth germ.

Initially the whole of the labial aspect of the dental lamina shows condensation of the underlying mesenchyme, but with specialization of the milk tooth area and retrogression of the more labial regions, condensation becomes restricted to the milk tooth area.

The significance of the 'wave pattern' of human dental growth is compared with the tooth formation in lower animals with continuous dental succession.

The Post natal fate of the abdominal para-aortic bodies in man.

By R. E. Coupland. *University of Leeds*

In the abdomen of the human foetus collections of extra-adrenal chromaffin cells (commonly referred to as para-aortic bodies or paraganglia) increase in size up to full-term.

The post-natal fate of the structures has been followed by fixing the abdominal aorta and adjacent tissues in formol-dichromate and making serial sections. Sections have been stained by Ehrlich's haematoxylin or iron haematoxylin or Giemsa. Graphic reconstructions have been made.

The findings indicate that the para-aortic bodies continue to increase in bulk up to 3 years and have a normal (late foetal) appearance at this time. In specimens aged 5 years and older degenerative changes are observed and are characterized by a marked increase in perivascular collagenous tissue and an apparent shrinking of the bodies. In specimens aged 14 and 22 years true para-aortic bodies no longer exist, although small collections of chromaffin cells may be seen. Extra-adrenal chromaffin cells were not observed in a specimen aged 49 years.

The findings indicate that degeneration begins in the child between the ages of 3 and

5 years. By the age of 14 years only a few scattered chromaffin cells are observed in sites originally occupied by the larger bodies; these are associated with relatively vascular connective tissue—the remains of the former bodies.

The attachment and shift of muscles. By N. B. B. Symons.

University of St Andrews, Dundee

It is generally accepted that during the period of skeletal growth the muscles shift relative to the bones and maintain their spatial relationships by means of a periosteal 'slip'. This implies either that the periosteum and the attached muscles are free to shift or that if a definite attachment of the muscles through periosteum to bone exists, then some mechanism exists to break this down. The shift must be particularly great in those muscles which are attached near the growing ends of a long bone. The attachment of the muscles to the upper half of the humerus and that of the lateral pterygoid to the condyle of the mandible which has an epiphyseal-like growth, have been examined in foetal material. All the muscles examined show a definite attachment to the underlying bone through fibre bundles which penetrate the periosteal layer and link-up with fibres passing out from the bone. Over part of the attachment area of a muscle, osteoclasts are to be found removing the bone to which the muscles are attached. This resorption, which brings about the normal modelling of bones during growth, frees the periosteum and muscles from the underlying bone, and would thus appear also to be concerned in muscle shift.

Notes on the regeneration of striated muscle in suckling mice.

By F. J. Aumonier. *Middlesex Hospital Medical School, London*

The degenerative changes produced in infant mice by infections of the Coxsackie group of viruses are briefly mentioned. In the 'A' group of viruses, the skeletal muscles are the only tissues involved, and this permits an experimental study to be made on animals whose muscles have been injured without any accompanying damage to other types of tissue.

It was found that the process of regeneration was similar to that described by Le Gros Clark for the limb muscles of the rabbit, but the earliest appearance of regeneration was much sooner than in the case of Le Gros Clark's material. The whole time sequence appears to be shorter, as is to be expected in a young animal. Branching of regenerating fibres is not so frequently found as in the adult rabbit, though this may be due to the greatly increased speed of the whole process. Melnick's statement that the regenerating muscles contain many more fibres than they did originally was not confirmed.

The first signs of regeneration can be seen in mice killed 48 hr. after the injection of the virus, and in specimens obtained from individuals sacrificed 56 hr. after infection it has reached a more pronounced development. After 14 days the new fibres are highly developed and show fully differentiated striations.

JUNE 1953

The Summer Meeting of the Society for the Session 1952–3 was held on Friday and Saturday, 10 and 11 July 1953, in the Anatomy Department, University of Liverpool. The President (Professor W. E. Le Gros Clark) was in the Chair.

The following are the authors' abstracts of the papers read.

The effect of ligation of the vasa efferentia on the rat testis.

By R. G. Harrison. *University of Liverpool*

Ligation of the vasa efferentia in the rat leads to enlargement of the testis within 24 hr.; the vasa efferentia distal to the site of obstruction are distended with spermatozoa, the rete testis is dilated and some spermatogonia are pyknotic or phytoid in appearance. By the seventh day after operation the testis has diminished in size, normal spermatogenesis is seen in only a few central seminiferous tubules, and all peripheral tubules show severe spermatogenic damage; fibroblastic invasion and proliferation of the lining epithelium of the vasa efferentia distal to the obstruction is occurring. Fourteen to sixteen days after operation the peripheral seminiferous tubules show spermatogenic destruction, and a few central tubules are at the stage of severe spermatogenic damage. After the tenth post-operative week the vasa efferentia distal to the site of ligation are irrevocably obstructed, the rete testis is still dilated, and all seminiferous tubules show spermatogenic destruction, only surviving Sertoli cells with grossly vacuolated cytoplasm being present; the Leydig cells are intact and there is oedema of the interstitium.

These changes are due only to obstruction to the efferent ductules of the testis, and not to operative manipulation, artificial cryptorchidism, or interference with the nerve supply, vascularization or lymphatic drainage of the testis.

The effects of higher epididymal obstructions upon the Leydig cells of the rat testis. By E. W. MACMILLAN. *University of Liverpool*

A previous communication (December, 1951) described the early effects of experimental arterial interruptions which occlude the main blood supply to the head and the adjoining portion of the body of the rat epididymus. A study of long-term material reveals that the epididymal lesion undergoes fibrosis, as do the efferential tubules, and the end result is an obstructive azoospermia. Testicular atrophy is still evident 11 months after operation.

Gross measurements at various postoperative intervals confirm that the volume of the testis on the operated (right) side is about one-third to one-half of that on the control side. Histologically the relative decrease in volume is manifest in the widespread degeneration of seminiferous tubules; on the other hand, the interstitial tissue appears to preserve its characteristic features.

The total absolute volume of Leydig cells in both the right and left testes was calculated by the paperweight method at periods ranging from 7 to 342 days after operation. A comparison between the readings shows that the total volume of the Leydig cells on the operated side is, as a rule, less than that on the control side. Nevertheless, in two animals sacrificed at 28 and 189 days respectively, there appears to be a volume increase, moderately so in the former and very markedly in the latter.

The readings at 7, 14, 56, 91, 226 and 342 days after operation argue against a compensatory hypertrophy; the findings at 28 and 189 days suggest that, under certain conditions, an absolute increase in the volume of Leydig cells may follow high epididymal obstructions initiated by experimental interruption of capital arteries in the rat.

The morphology of the collateral circulation following complete interruption of the abdominal aorta in the rat. By J. L. BRAITHWAITE. *University of Liverpool*

The origins of the branches of the abdominal aorta have been studied in twenty animals and ligation experiments have been carried out in thirty-six. The sites of interruption varied between the origin of the coeliac artery and a point just proximal to the aortic bifurcation. In the twenty-nine animals surviving operation the morphology of the collateral circulation was studied by sacrificing the rats at periods up to three months after operation. Radiographic and dissection techniques were employed to elucidate the results. Ensuing complications were also noted.

The results demonstrate that the abdominal aorta can be ligated with comparative safety at all levels distal to the origin of the left renal artery—all the fatalities except one following more proximal interruptions. The macroscopic collateral channels (both visceral and parietal) became noticeably enlarged after a period of about four days, although the degree of enlargement of these varied with the site of ligation. The most important anastomotic channels were the spermatic, mesenteric, ureteric, interlumbar and those occurring in the body wall. Additionally, a more localized plexus of fine vessels was manifest in the region of the ligation site.

The main complication following these procedures was a temporary paralysis of the hind extremities lasting from 2 to 3 days, although necrosis of the tail occurred in two instances, and gangrene of the left uterine horn in one.

The vascularization of the thyroid gland. By T. D. Reilly, *University of Liverpool.* (Introduced by R. G. Harrison)

The arterial supply of the human thyroid gland has been investigated in eleven normal post-mortem specimens, by the injection of bismuth oxychloride suspension. The anastomosis between individual thyroid arteries is often less than arterial size. In two cases the cricothyroid artery is very large and passes across the cricothyroid membrane to vascularize the isthmus.

In the rabbit two arteries which correspond to branches of the superior thyroid artery in man supply each side of the thyroid gland. These arteries were ligated in seven rabbits and the arterial supply observed post-operatively following the injection of 'Micropaque'. The animals were sacrificed at intervals of 1, 2 and 3 weeks, and in every case each side of the gland was now found to be supplied by an artery formed by the opening up of an anastomosis between the tracheal branch of the thyroid artery and the tracheal branch of the bronchial artery. Histologically the gland showed no significant change, and this was confirmed by the normal growth rate of operated as compared with control animals.

In one human thyroid gland examined 2 months after partial thyroidectomy in which one inferior thyroid artery and both superior thyroid arteries were ligated, the remainder of the gland was vascularized by a vessel formed by anastomosis of the superior laryngeal branch of the superior thyroid artery with the inferior laryngeal branch of the inferior thyroid artery, the tracheal branch of the latter being markedly dilated.

The absorption of triolein from the small intestine of the rat. By W. Hewitt. *University of Liverpool*

During the last half century, it has been generally accepted that triglyceride fat passes through the epithelial cells of the mucosa when it is absorbed from the lumen of the small intestine. Prior to this it has been considered, by certain authors, to pass between these cells. It is at present debatable whether some of the fat is absorbed in an unhydrolysed state.

By using histochemical techniques, studies have been made on the small intestine of rats after feeding with triolein. These observations demonstrate that neutral fat can be seen passing between the epithelial cells and that the material accumulating within the cells is fatty acid. These results therefore provide support for the hypothesis that during the absorption of triglyceride fat some of it is absorbed in an unhydrolysed condition.

The arterial supply of the human prostate and seminal vesicles. By E. J. Clegg. *University of Liverpool.* (Introduced by R. G. Harrison)

The arterial supply of the prostate and seminal vesicles has been studied in a total of twenty-one half pelves by radiography and dissection.

The prostate gland is constantly supplied by the prostatic artery, a branch of the prostato-vesical trunk. As a rule, the distribution is to both surfaces of the gland, but the supply to the posterior surface may be deficient when the gland is supplied on this aspect by the posterior vesicular (author's terminology) or vesiculo-deferential arteries. The superior rectal artery supplies the gland in 32·1 % of cases, a high figure compared with Awataguti's (1939) finding of 14·3 %.

The seminal vesicle is constantly supplied on its anterior aspect by the anterior vesicular artery (author's terminology), a branch of the vesiculo-deferential artery. Its posterior aspect is supplied by the posterior vesicular artery, which may be a branch of the prostato-vesical, the gluteo-pudendal, or rarely, the vesiculo-deferential artery.

It is considered that variations in the nomenclature of blood vessels in this region account, in the main, for the wide diversity of findings in the blood supply of the prostate and seminal vesicle.

Some anatomical factors concerned in the regulation of C.S.F. pressure.

By D. R. Bowsher. *University of Liverpool.* (Introduced by R. G. Harrison)

Retrograde injection of a radio-opaque barium suspension into the azygos vein of man, monkey, dog, cat, rabbit and rat, has shown that the main connexions of the azygos vein are with the intra- and extra-dural vertebral veins. Physiological increase in C.S.F. pressure is due to an increase in volume of these intra-dural veins (Bowsher, *Brit. med. J.* 1953, **1**, 863). In the closed thecal sac this volume increase is compensated by increased amount of C.S.F. into these veins, and thus a removal of an equivalent amount of C.S.F. from the spinal theca. This has been demonstrated by two methods:

(*a*) In a model in which a semipermeable elastic tube runs through a water-jacket, whose pressure is sufficient almost to collapse the inner tube. When a flow is initiated through the semipermeable tube, it expands to assume a circular cross-section. Chemical substances introduced into the water-jacket can be recovered from the outflow.

(*b*) When radioactive sodium (^{24}Na) is introduced into the subarachnoid space of a living animal, the substance is absorbed into the blood at a regular rate. When C.S.F. pressure is raised by compression of the abdominal vena cava, the rate of uptake is immediately accelerated, but returns to its original level before the compression is released, indicating that C.S.F. pressure equilibrium has been restored by the removal of an appropriate quantity of C.S.F. from the subarachnoid space.

Retrograde cell degeneration following lesions of the fornix system.

By H. M. Daitz and T. P. S. Powell. *University of Oxford*

The retrograde cell degeneration following lesions of the fornix system has been studied in the rat, rabbit and macaque monkey. In no case was any cellular change found in the hippocampus, irrespective of the age of the animal or the survival time. Marked retrograde degeneration consistently occurred in the medial septal nucleus and the nucleus of the diagonal band on the operated side. These nuclei showed no change after extensive destruction of the entorhinal cortex and amygdaloid nuclei. No cellular degeneration was found in the preoptic and hypothalamic areas. Following fimbrial lesions in the rat the alveus showed fibre loss; the distribution of the latter indicates that field CA$_1$ of the hippocampus is related to the dorsal fornix and the other fields to the fimbria.

Phase contrast observations of the process of haemolysis in salamander erythrocytes. By W. D. Trotter. *University of Oxford*

There is little information available in the literature on the microscopic aspects of haemolysis in nucleated red cells. The large size of salamander erythrocytes makes them especially suitable for studying the visible changes accompanying haemolysis. The cells were haemolysed by a variety of agents including water, acids, alkalis, saponin, digitonin, bile salts, soaps, anionic and cationic detergents. Haemolysis was accompanied by changes in the optical properties, the size and the shape of the cell as a whole, and in the shape and optical appearance of the nucleus. The appearances varied with the method of producing haemolysis.

Alkalis produced bizarre shape changes followed by sphering of the distorted cells which then underwent rapid complete cytolysis. Water gave spherical prolytic cells, the ghosts being either circular or recovering an elliptical outline, and in many cases showing partial or complete extrusion of the nucleus.

Acids produced grossly distended ghosts exhibiting an apparently rigid fibrillar structure in the part of the 'membrane' forming the original rim of the cell.

The majority of natural and synthetic surface active agents gave ghosts of elliptical outline when used in high concentration, and a large proportion of circular ghosts with weaker concentrations. With some lysins haemolysis was succeeded by gross swelling and final dissolution of the nucleus.

The relationship of these and various other changes to theories of red cell structure were discussed.

An ovarian pregnancy. By W. J. HAMILTON and AILEEN M. DICKINS.

Charing Cross Hospital Medical School, London

The possibility of the occurrence of an ovarian pregnancy was first suggested by Mercer in 1614. It was not, however, until 1899 that careful histological description of an early case was published by Catherine Van Tussenbeck. She demonstrated that the ovum could be implanted in the ovary. Since that date more than one hundred specimens of this condition have been described. Many of the cases may not be 'true ovarian' pregnancies. The present specimen was obtained from a woman aged 18. She was delivered in August 1951 of a normal child. Since the birth of this child the menstrual cycle was of thirty-days type and the menstrual period lasted for 7 days. She had a normal period on 24–31 October and again on 23–30 November 1952. On 3 December she felt a colicky pain in the lower abdomen which lasted a short time. This pain again occurred on 9 December; it became more severe on 10 December and the patient was admitted to hospital.

At laparotomy (12 December 1952) there was a little blood in the peritoneal cavity, the uterus was soft and bulky, and both Fallopian tubes and the left ovary were perfectly normal and free from blood clot. The right ovary was enveloped in clot (but this did not involve the associated tube). The clot could be peeled from most of the ovary except its medial pole. The ovarian and infundibulo-pelvic ligaments could be readily seen in their normal relations to the ovary. A wedge resection of two-thirds of the affected ovary was carried out. The patient's recovery was uneventful.

The ovary was fixed and later transferred to the laboratory.

When part of the clot was removed from the ovary it became apparent that in the clot there was a rough walled vesicle. The vesicle and ovary were then divided into two. On being sectioned the vesicle was found to be attached to the surface of the ovary at the site of a well-developed corpus luteum. A somite embryo with a head and a tail fold was found. The secondary chorionic villi were present over the entire surface of the vesicle but were better developed at their site of attachment to the ovary. The corpus luteum showed signs of retrogression.

Film on living human blood cells. By N. H. HANCOX.

University of Liverpool

This film was prepared for teaching purposes. Time lapse micro-cinephotography was employed and both phase contrast and darkfield illumination were utilized.

The general appearance and movements of normal living erythrocytes, neutrophils, eosinophils and lymphocytes are shown.

Early stages in the development of the placenta of *Dama dama* L. By R. J. HARRISON and A. R. HYETT. *London Hospital Medical College*

Nearly three hundred specimens of the non-pregnant and pregnant uterus have been collected in conjunction with Charing Cross Hospital Medical School. Examinations of this series, and observations made in the field, show that mating occurs throughout October and early November in southern England, and that parturition takes place between the middle of May and the middle of June. Young does can become pregnant at the age of

15–18 months; there is no evidence of delayed implantation. Twinning is rare, only one instance being recorded in this series.

The caruncles in the young non-pregnant uterus often have a leaf-like form; later they become sessile ovoids. There are commonly four caruncles in each horn. In the earliest stages recovered the chorionic sac had already extended into both horns; there was no evidence of development of villi in these specimens, nor in any in which the embryo was between 1 and 8 mm. in greatest length. Villi are present in the cotyledonary 'plaques' at the 12–15 mm. C.R. stage; each plaque lies opposite a caruncle. The villi develop in long, distinct rows, the majority running parallel to one another and transverse to the long axis of the uterine horn. The rows of villi are equidistant from each other, as are the individual villi in a row, except at the edges of the plaque, where growth of new villi occurs. The linear distribution of the villi bears no relation to the pattern of the blood vessels supplying the developing cotyledon. The number of villi in each cotyledonary plaque has been counted. Increase in the number of villi occurs to some extent by addition at the edges of a plaque, but also by branching of individual villi. The crypts in the maternal caruncle also first appear in rows lying transverse to the long axis of the caruncle. Each row of crypts is separated from its neighbours by a raised ridge of caruncular tissue. As the placentome grows the linear arrangement of villi and crypts is lost and is replaced by a polygonal pattern at about the 150 mm. C.R. stage. The changes in shape of the growing placentome and its villi were described, and estimations made of the numbers of the villi and crypts at different stages of growth.

The early embryology of the Emperor Penguin. By T. W. GLENISTER. *Charing Cross Hospital Medical School, London*

Sixteen Emperor Penguin embryos collected by Stonehouse of the Falkland Island Dependencies Survey constitute the first series to contain early stages. The eggs were collected in the Antarctic Winter of 1949 off Dion Island. The embryos have been compared with embryos of other species of penguin and chick embryos. The comparison reveals that early penguin embryos and especially Emperor Penguin embryos, resemble early reptilian embryos more closely than do early chick embryos by having a relatively smaller head, a relatively longer neck and tail, and less well-marked curvatures. The younger embryos possess well-developed head cavities, epibranchial placodes and neurenteric canal. The transient appearance of an epithelial proliferation in the dorsal portion of the second visceral pouch probably represents the thymus II of lower vertebrates. An independent curve in the bulbus arteriosus of some of the early embryos is similar to that described in crocodilian embryos. As in embryos of other species of penguin, incipient dorsal air sacs develop from the ectobronchi. The Emperor Penguin also develops a pronephros which resembles in some respects that of reptilian embryos. These features suggest that Wilson was right in stating that possibly the Emperor Penguin is the most primitive form of bird we have living to-day.

Closure of the pleuro-peritoneal openings in human and goat embryos. By W. R. M. MORTON. *The Queen's University, Belfast*

Serial sections (transverse and sagittal) of a graded series of twenty human embryos, ranging from 4 mm. C.R. to 43 mm. C.R. in length, were examined to determine the sequence of events leading to the closure of the opening between the pleural and general peritoneal cavities. A similar series of fifteen goat embryos (21 days to 38 mm. C.R.) was also examined for comparison. The method of closure of the opening in the human differs on the two sides of the body. The covering membrane on the lateral side of the pneumato-enteric recess effects an early junction between the dorsal capsule of the liver, the dorsal mesentery of the foregut and the right suprarenal, to close the opening medially. The very early disappearance of the coelomic niche on the left side of the foregut and the expansion of the stomach to the left cause later closure of the medial part of the opening and this differs in detail from that on the right side. The liver capsule effects only a slight attachment to the dorsal mesentery, and the latter alone (at this stage) makes an attachment to the antero-medial aspect of the upper pole of the left suprarenal. On both sides the developing pleural coelome changes its position relative to the suprarenals from antero-medial to postero-lateral by passing medially around the upper pole of the suprarenal.

Final closure of the pleuro-peritoneal openings appears to be due to proliferation of the cells around the now small slit-like openings between the dorsal extensions of the liver and the pleuro-peritoneal membranes. The latter are attached posteriorly (as the dorsal pillars of Uskow) medial to the mesonephros, and as this degenerates the dorsal attachments merge with the suspensory ligaments.

Neither the cutting off of the infra-cardiac bursa from the superior recess, nor the final closure of the pleuro-peritoneal openings is by growth of muscle fibres.

In the goat the liver joins the mesonephros, which is large and persists longer than in the human, and the final closure of the pleuro-peritoneal canals is delayed till after the 35 mm. C.R. stage.

Epiphyseal excision and transplantation in the rabbit. By P. A. RING. *Charing Cross Hospital Medical School, London*

A series of experiments has been performed upon the distal ulnar epiphyseal cartilage of the rabbit to determine its reaction to partial and complete excision and to autogenous transplantation. The following conclusions have been reached:

(1) Simple excision of the whole cartilaginous area with a small area of bone of the metaphysis and epiphysis is followed by bony union between the remainder of the epiphysis and the shaft. There is no histological or radiological evidence of regeneration.

(2) The important area of the epiphysis is that part in which the cartilage cells are dividing. Excision of this area stops further growth; excision of the degenerating cartilage has no effect.

(3) Simple excision and replacement of the epiphyseal cartilage in its original position is followed by normal growth. Occasional failures may be due to non-union of the fragments, or to ossification in the haematoma.

(4) If the same area is replaced in the reversed position, the cartilage retains its polarity, and a large epiphyseal plate of bone is formed. This is followed by premature fusion of the epiphysis. Cross union between radius and ulna may retard growth, although commonly this merely alters the structure of the elbow joint.

(5) Damage or removal of the metaphysis during these procedures does not affect the growth pattern produced, provided the graft unites.

Observations on the oesophageal hiatus of the diaphragm with a description of a case of oesophageal hiatus hernia. By W. K. J. WALLS. *University of Leeds*

The arrangement of muscle in the neighbourhood of the oesophageal hiatus of the diaphragm is subject to considerable variation. Low (1907) described the oesophageal passage in the diaphragm as an elongated slit formed by the splitting of the mesial fibres of the right crus. The findings in the present investigation, based on the dissection of over forty cases, in general confirm the views of Low, but with some differences.

The findings are summarized as follows:

(1) In almost all cases the right crus of the diaphragm plays the predominant part in forming the margins of the oesophageal hiatus.

(2) In about three-quarters of the cases the right crus alone forms the margins of the hiatus, but (and this is at variance with Low), in about one-quarter of the cases the left crus forms some part of the right margin of the orifice.

(3) In almost half the cases a band from the left crus crosses to the right of the oesophageal hiatus on its way to the central tendon near the caval orifice, but, except when the hiatus is enlarged, forms no part of its margin.

The findings were illustrated by lantern slides.

A case of oesophageal hiatus hernia, found incidentally, was described.

The attachments of the metatarsal heads. By R. Wheeler Haines. *University of Sheffield*

The heads of the metatarsals have no direct attachments to each other, but are attached by ligaments to the plantar pads, and these pads are bound together by the deep transverse ligaments of the sole. The two sesamoids developed in the first pad articulate with grooves on the plantar surface of the first metatarsal head. When in hallux valgus or metatarsus quintus varus the metatarsals become spread, the deep transverse ligaments are not, as has often been suggested on theoretical grounds, unduly stretched: it is the ligaments that bind the pads to the metatarsal heads that in fact give way, while the sesamoid mechanism of the first joint is destroyed. The first plantar metatarsal artery has a tortuous course passing between the two heads of the flexor hallucis brevis and then proximal to the lateral sesamoid, so that it is liable to occlusion when the parts are displaced.

The attachments of the masseter muscle. By J. D. B. MacDougall. *University College, Dundee*

Recent papers on the attachments of the masseter muscle tend to confirm a statement made by Winslow in 1732 that the muscle has three distinct heads instead of a superficial and a deep head as is usually described. There is still disagreement, however, among authors as to the precise extent of each of these parts.

In an attempt to clarify the position, the present study was based on dissections of 141 human masseter muscles, on the histological examination of eight foetal and two adult lower jaws, and on the injection of barium sulphate paste into the masseter in five intact cadavers and their subsequent X-ray and dissection. The attachment of the masseter to the ramus of the mandible was found to be firm in the region of the coronoid process and near the angle of the jaw, but loose elsewhere, although continuous, leaving no bare areas on the bone. An attempt has been made to correlate this description with a recent account of the development of the mandible.

Two planes of cleavage were demonstrated in the muscle, both by dissection and by histological examination, resulting in the appearance of three heads, a superficial, a middle and a deep.

The attachments of these three heads, their relationship to the vessels and nerves of the masseter, and their significance in relation to the spread of infection was discussed.

Electromyography of postural muscles—leg and thigh. By J. Joseph. *Guy's Hospital Medical School, London*

In a previous communication, it was suggested that in the stand-at-ease position the soleus was constantly active and the tibialis anterior inactive. In a further study of twelve males and twelve females, this was confirmed. In the case of the thigh muscles, the same position was maintained without any apparent activity in the quadriceps extensor and hamstring muscles. These results were not affected by unconscious swaying. It is suggested that the line of weight falls in front of the centre of the knee joint in the stand-at-ease position and the appropriate ligaments of the knee joint bear the weight of the body. They only do so to a limited extent, however, as is shown by voluntary swaying forwards and moving the body weight by raising the arms forwards, since in both of these activities the hamstrings become active.

These findings were demonstrated by means of a sound film.

An electromyographic study of certain muscles of the leg and of the foot in the standing position. By J. V. Basmajian and J. W. Bentzon. *University of Toronto*

Using unipolar needle electrodes paired with skin electrodes, an electromyographic study was made of three muscles of the leg (tibialis anterior, peroneus longus, and the lateral head of gastrocnemius) and three intrinsic muscles of the foot (abductor hallucis, abductor digiti quinti, and flexor digitorum brevis). The findings during relaxed standing at ease were of special interest. The muscles of the leg were studied in seventeen men and sixteen women, and those of the foot in a total of twelve persons.

An analysis of the experimental data shows that tibialis anterior, peroneus longus, and the intrinsic muscles of the foot were electromyographically inactive in many subjects, suggesting that these muscles play no important role in maintaining the arches of the foot during relaxed standing at ease.

All six muscles showed a wide biological range of activity from person to person rather than a single type of activity in all persons. In the men, gastrocnemius showed by far the greatest activity. In the women, this difference was not seen when the subjects were barefooted. When high heels were worn, gastrocnemius and peroneus longus, but not tibialis anterior, showed a marked increase in activity in most women. A possible explanation is that the centre of gravity shifts forward when high heels are worn.

There was no obvious relationship between electromyographic findings and types of feet.

Some observations on the venous systems in the pinnipedia. By J. D. W. Tomlinson and R. J. Harrison. *London Hospital Medical College*

The arrangement of a number of veins has been examined in twenty adult specimens of *Phoca vitulina*, twelve new-born pups of *Halichoerus grypus* and in foetuses of *Leptonychotes weddelli*, *Lobodon carcinophagus* and *Zalophus californianus*. In many specimens the adult vascular system was injected with coloured gelatin or coloured resin with subsequent digestion in acid.

The majority of the renal veins are arranged in a network over the surface of the kidney lying between its many lobules. This network is drained by three main veins with their associated complicated venous plexuses. The veins drain medially into large paired postcaval vessels which unite at the upper poles of the kidneys into a single vein. The latter enters a large venous reservoir, the hepatic sinus of Burne (1910), which can contain at least 600 ml. of blood in *Phoca vitulina*. The short inferior vena cava has a well-developed muscular sphincter arranged around it just above the diaphragm.

Large anastomotic veins pass from the posterior renal plexus through the inter-vertebral foramina to form a large venous sinus within the spinal canal. This sinus lies dorsal to the spinal cord and runs the whole length of the spinal column. The sinus divides in the cervical region in *P. vitulina* and passes upwards into the skull to communicate with the cranial dural sinuses through the condylar canal. The tributaries of the vein in the spinal canal were described and the possible functions of the numerous venous anastomotic channels in the seal discussed.

The comparative anatomy and function of the maxillary turbinate. By J. H. Scott. *The Queen's University, Belfast*

The inferior (maxillary) turbinate develops from the cartilage of the nasal capsule by endochondral ossification. Among mammals it is absent in cetaceans; is simple in form among the higher primates and complex in many carnivores and some rodents. It reaches its highest complexity in seals and sea-lions.

In animals in which the maxillary turbinates are complex the bony skeleton consists of numerous secondary and tertiary laminae, while the covering mucous membrane has a surface area in the nasal cavity greater than the skin surface of the body. The mucous membrane is highly vascular with many large sinus-like spaces close to the surface. The epithelium contains numerous mucus-secreting cells.

Complex turbinates are found in animals which lead an active life and in which the skin is loaded with fat (seals) or covered with dense hair and in which sweat glands are absent. It is unlikely that the chief function of the mucous membrane is the warming of inspired air, as many animals with complex turbinates live in temperate or tropical climates, and in many animals the air-warming function is performed by a much less extensive mucous surface (ungulates). It is suggested that the primary function of the mucous membrane is control of body temperature through regulation of the loss of body heat, a function which in the higher primates and to a certain extent in many ungulates is transferred to the skin. In man the skin is the chief organ of temperature control and the maxillary turbinate has undergone a secondary degeneration.

The anatomy of dephosphorylation. By G. H. Bourne.

London Hospital Medical College

Dephosphorylating enzymes are widely distributed in the animal body. In soft tissues they are present in kidney, alimentary tract, brain membranes, capillaries and some small blood vessels, in the areolar coats of many large vessels and in polymorpho nuclear leucocytes. In bone they are present in both osteoblasts and osteoclasts, in hypertrophied cartilage cells, in periosteal and endosteal cells, and in bone marrow. In the repair of bone or soft tissues the first-formed fibrous tissue is rich in phosphatase which disappears as this tissue matures. The role of phosphatase in bone formation has been discussed a number of times, but its role in soft tissues is not so obvious. However, it is noteworthy that there is a high concentration of phosphatase activity in membranes where rapid passage of substances from one side to the other is important; for example, the only tissues in the brain which give a positive reaction at all are the capillaries and the brain membranes. Also, in absorbing surfaces such as those of the small intestine and the kidney tubules there is quite strong evidence that the phosphatase is located at the brush borders of cells. In general the distribution of phosphorolytic activity in the soft tissues seems to be associated with the problem of membrane permeability.

Although we do not know the precise mechanism whereby dephosphorylation results in penetration of a membrane by various substances, it is known that some phosphate bonds, when broken, release energy and this energy could presumably be used to drive substances across the membrane. A study by the author of the ability of the body to dephosphorylate a number of sugar phosphates has shown that they all appear capable of dephosphorylation by non-specific alkaline phosphatase, an exception being sorbose phosphate which is not a naturally occurring phosphate. The distribution of the enzymes which dephosphorylate aneurin (vitamin B_1) pyrophosphate, pyridoxine phosphate and riboflavin phosphate, which are themselves either enzymes or prosthetic groups of enzymes, is similar to that of non-specific phosphatase in some organs (where their passage through a membrane is important) and not in others. The body also contains enzymes which dephosphorylate oestrogen phosphates. It is not known whether oestrogens are phosphorylated *in vivo*, but it is of interest that certain cells in the brain can dephosphorylate such compounds. It is also of interest that the walls of the sinusoids of the adrenal cortex which contain enzymes capable of readily dephosphorylating sugar and vitamin phosphates have no ability to dephosphorylate oestrogen phosphates. Perhaps absence of activity in this instance is an example of a protective mechanism whereby adrenal cortex cells are themselves protected against undue influence by oestrogens.

Impressiones cisternales. By Jelena Krmpotić. *University of Zagreb*

In 7 % of human occipital bones, well-marked smooth depressions are seen lateral to the internal occipital crest. These depressions, which may be unilateral or bilateral, vary in shape and size; they may be oval or roundish in contour. The greatest length seen for one of these depressions was 20 mm. and the greatest depth 9 mm. They are related to the arachnoidal sacs, which are lateral continuations of the cisterna magna. It is suggested therefore that the depressions be named 'impressiones cisternales' and the arachnoidal sacs, 'recessus cisternales'.

The so-called nucleus of convergence. By R. WARWICK. *University of Manchester*

Shortly after Perlia (1889) described a midline group of nerve cells, somatic in type, in the human oculomotor complex, Knies (1891) ascribed to it, largely on clinical grounds, the function of convergence. This concept received considerable approval from neurologists and ophthalmologists, and it was emphasized particularly by Brouwer (1918), who equated the phylogenetic emergence of the nucleus of Perlia, as a topographical entity, with the development of binocular vision.

Despite serious criticisms, especially by Tsuchida (1906) and Le Gros Clark, chiefly concerning the comparative anatomy of the central nucleus, its supposed role as mediator of convergence movements of the eyes has been accepted, apparently, in most standard accounts up to the present day. Moreover, the rationale of Stutterheim's system of orthoptics depends basically upon this concept.

Experimental and topographical data, collected from examination of eighty monkey midbrains, show that in this animal Perlia's nucleus is a doubtful entity and does not control convergence.

'Toeing-in'. By J. McKENZIE. *University of Aberdeen*

Elftman (*Anat. Rec.* 59, 1934), states that the inner border of the heel bears the greater weight in individuals who walk 'toeing-in'. In fourteen healthy adults who walk 'toeing-in' with one or both feet, it is in each case the lateral side of the heel of the shoe which is worn down.

In these people the weight is borne along the whole length of the lateral part of the foot and 'toeing-in' is the result of inverting the foot in order to 'push off' with the lateral toes.

A cinematograph film was shown to demonstrate this mechanism.

Variations in leucocyte counts during the human menstrual cycle.
By I. L. MACKINNON. *King's College, London*

Total leucocyte counts were recorded daily from one healthy unmarried woman, aged 25, under basal conditions, during a period of a year incorporating thirteen menstrual cycles.

The average total leucocyte count for each day of the menstrual cycle was calculated and the counts examined statistically. It was found that they were significantly higher on the 22nd–24th days than on the 4th–6th days of the cycle. The possible causes—body temperature, sex hormones and suprarenal cortical hormones—were discussed.

Healing of mucosal lesions of the small intestine of the cat.
By R. M. H. McMINN and J. E. MITCHELL. *University of Sheffield*

The healing of mucosal lesions of the small intestine has been studied in the cat. At a site 18 in. proximal to the ileo-caecal junction an 'artificial ulcer' was created by the removal of 1 sq.cm. of mucosa and submucosa. The animals were killed at varying periods after operation and the site of the lesion was examined histologically. The results show that not only is epithelialization complete but that the tissues underlying the epithelium have taken part in a reparative process which restores the mucosal architecture to a state closely resembling the normal. The precursors of crypts and villi are seen as early as the fourth post-operative day at the periphery of the ulcer, and this crypt and villus formation gradually proceeds towards the centre. By the end of 3 months the distinction between normal and reconstituted mucosa is indicated only by the absence of muscularis mucosae and by varying amounts of fibrous tissue in the position normally occupied by the submucosa.

Preliminary observations of mitotic activity in the regenerating epithelium using colchicine (0·25 mg. per kilogram of body weight given intraperitoneally 5 hr. before death) suggest that mitosis is more frequent 24 hr. after operation than at other times.

The distribution of taste buds on the human epiglottis.

By G. J. Romanes. *University of Edinburgh*

In children and adults numerous taste buds are found confined to the posterior surface of the epiglottis. Though scattered throughout they tend to be arranged in two main groups close to a marginal strip of epithelium devoid of taste buds and much thicker than in the bud-bearing areas. These two richly innervated groups face the arytenoid cartilages whose antero-lateral surfaces are likewise covered with taste buds. It is considered that the adjacent surfaces of the epiglottis and arytenoid cartilages are closely applied during deglutition so that their taste buds are in apposition and not directly exposed to food. The thin epithelium and the exposed nature of the taste buds are in keeping with this view.

Studies on cat, rat, guinea-pig, mole and hedgehog have failed to reveal a comparable number of taste buds on the epiglottis, though a few may be found in the mole on the anterior as well as the posterior surface, the latter being more usual.

Histochemical demonstration of amine oxidase.

By H. Blaschko and K. Hellmann. *University of Oxford*

An attempt at a histochemical reaction for amine oxidase was made by Oster & Schlossman (1942), but this has not been found satisfactory. The basis of our method is the enzymic oxidation of tryptamine as the result of which a coloured compound is formed. A similar pigment formation occurs when 5-hydroxytryptamine (serotonin) is oxidized by tissue preparations containing amine oxidase. Tissue sections were incubated with tryptamine or serotonin, and the formation and distribution of pigment studied.

Fixatives such as formol-saline, acetone and alcohol reduce the pigment formation to negligible amounts even when the tissues are exposed to them for only a short period. Fresh material was therefore always used. It was cut on a freezing microtome at 25μ thickness and the sections incubated in 0·067 M-sodium phosphate buffer of pH 7·4 at 37° C. In most of our experiments tryptamine hydrochloride in a concentration of 1 mg./ml. was used as a substrate for amine oxidase activity. These conditions were found to be optimal for pigment production. The addition of small quantities of hydrogen peroxide was found to speed up pigment production considerably. Of inhibitors tested, pentamidine was found to be the most effective. In a concentration of 10^{-5} M it abolished pigment formation.

The distribution of this tryptamine reaction in different tissues followed the distribution of amine oxidase as known from biochemical experiments.

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